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Serology of Immunity

IMMUNITY

PROTECTIVE INOCULATIONS IN INFECTIOUS DISEASES

AND

SERUM-THERAPY

BY

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PREFACE.

The following quotations from a paper, entitled "Practical Results of Bacteriological Researches," read by the writer at the meeting of the Association of American Physicians, May 24, 1892, may serve as a preface to the present volume:

"Science does not demand practical results, but investigates for the purpose of establishing facts and explaining phenomena. And bacteriology, as a branch of natural history, is entitled to equal consideration with other departments of scientific research. Indeed, the low organisms known as bacteria offer many advantages for the study of fundamental biological problems, and the researches already made have borne abundant fruit from a scientific point of view.

"But medicine is eminently practical in its aims, and practising physicians, as well as intelligent laymen, are apt to meet every announcement of a new discovery in pathology with the question, 'Does it aid in the cure of disease?' Heretofore the bacteriologist has been compelled to admit that the demonstration of the specific cause in a considerable number of infectious diseases, which has been obtained through his researches, has not resulted in the discovery of a specific treatment for these diseases. At the present moment we are in possession of experimental data which open up to us a vista of possibilities in specific treatment unsuspected a year or two ago.

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"The researches of bacteriologists have established the fact that the pathogenic action of those bacteria which have been shown to be concerned in the etiology of specific infectious diseases is due to the formation of toxic products during the active development of the bacterial cells. The discovery of these toxins and toxalbumins has led to a line of research work, the object of which is to isolate and study each of these toxic products separately by the methods of chemistry and of experimental pathology.

"In the course of these experiments, and of the extended researches which have been made with reference to the explanation of natural and acquired immunity, the remarkable discoveries which will occupy our special attention have been made. . . .

"Evidently the production of an antitoxin during an attack of any one of the infectious diseases would account for recovery in non-fatal cases; and it may be that this is the true explanation of self-limitation in diseases of this class. If Nature adopts this method of cure, we but follow her if we seek to introduce more of the antitoxin for the purpose of arresting the progress of cases of unusual severity and fatal tendency. . . .

"Although the production of these antitoxins in considerable amounts for therapeutic use will be attended with difficulties, there can be no doubt that methods will be devised for obtaining them on a large scale as soon as it is definitely established that they may be successfully used as specifics in the treatment of infectious diseases.

"In diseases which are common to man and the lower animals the source from which they may be obtained is evident; but in diseases peculiar to man we do not at present see just how they are to be obtained. Reasoning

from the analogy afforded by the experimental evidence heretofore referred to, we infer that the blood and tissue juices of an individual who has recently suffered an attack of small-pox or scarlet fever contains an antitoxin which would neutralize the active poison of the disease in the circulation of another person immediately after infection. Whether a small quantity of blood drawn from the veins of the protected individual would suffice to arrest the progress of the diseases mentioned, or to modify their course, can only be decided by experiment; but the experiment seems to me to be a legitimate one. Possibly transfusion of a moderate amount of blood from one to the other might prove to be curative, or, if made in advance of infection, might confer immunity. It may be that an antitoxin can be obtained from the blood of vaccinated calves which would have a curative action in small-pox."

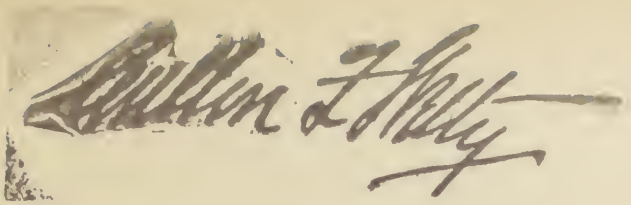
In the present volume the author has endeavored to give a summary of the most important experimental evidence in the field of research to which it relates. Comparatively little space has been given to the discussion of unsolved questions connected with the subject; nor has it been possible to review the entire literature; but the most important results of experiments made by competent bacteriologists have been stated as concisely as possible. In the bibliography the titles of many valuable papers will be found to which no reference has been made in the text.

As my official duties occupy the greater portion of my time I have worked under a certain disadvantage, but it has been a labor of love, and I trust that the result will prove to be acceptable to those members of the profession who wish to keep abreast of the progress of scien-

tific medicine and who have not the foreign literature at hand, or are too busy to make use of it.

In many places throughout the work I have introduced portions of the text of my "Manual of Bacteriology," * and of some of my recently published papers, without the use of quotation marks.

* William Wood & Co., New York, 1893.



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PART FIRST.

SUSCEPTIBILITY AND IMMUNITY.

I.

NATURAL IMMUNITY.

No questions in general biology are more interesting, or more important, from a practical point of view, than those which relate to the susceptibility of certain animals to the pathogenic action of certain species of bacteria, and the immunity, natural or acquired, from such pathogenic action which is possessed by other animals. It has long been known that certain infectious diseases, now demonstrated to be of bacterial origin, prevail only or principally among animals of a single species. Thus typhoid fever, cholera, and relapsing fever are diseases of man, and the lower animals do not suffer from them when they are prevailing as an epidemic. On the other hand, man has an immunity from many of the infectious diseases of the lower animals, and diseases of this class which prevail among animals are frequently limited to a single species. Again, several species, including man, may be susceptible to a disease, while other animals have a natural immunity from it. Thus tuberculosis is common to man, to cattle, to apes, and to small herbivorous animals by inoculation, while the carnivora are, as a rule, immune ; anthrax may be communicated by inoculation to man, to cattle, to sheep, to guinea-pigs, rabbits, and mice, but the rat, the dog, carnivorous animals, and birds are generally immune ; glanders, which is essentially a disease of the equine genus, may be communicated to man,

to the guinea-pig, and to field-mice, while house-mice, rabbits, cattle, and swine are to a great extent immune.

In addition to this general race immunity or susceptibility we have individual differences in susceptibility or resistance to the action of pathogenic bacteria, which may be either *natural* or *acquired*. As a rule, *young animals are more susceptible than older ones*. Thus in man the young are especially susceptible to scarlet fever, whooping-cough and other "children's diseases," and after forty years of age the susceptibility to tubercular infection is very much diminished. Among the lower animals it is a matter of common laboratory experience that the very young of susceptible species may be infected when inoculated with an "attenuated culture" which older animals of the same species are able to resist.

Considerable differences as to susceptibility may also exist among adults of the same species. In man these differences in individual susceptibility to infectious diseases are frequently manifested. Of a number of persons exposed to infection in the same way some may escape entirely, while others have attacks differing in severity and duration. In our experiments upon the lower animals we constantly meet with similar results, some individuals proving to be exceptionally resistant. Exceptional susceptibility or immunity may also be to some extent a family characteristic or one of race. Thus the negro race is decidedly less subject to yellow fever than the white race, and this disease is more fatal among the fair-skinned races living in the north of Europe than among the Latin races living in tropical or subtropical regions. On the other hand, small-pox appears to be exceptionally fatal among negroes.

A very remarkable instance of race immunity is that of Algerian sheep against anthrax, a disease which is very fatal to other sheep.

In the instances mentioned *race immunity* is probably an acquired tolerance due to natural selection and inheritance. If, for example, a susceptible population is exposed to the ravages of small-pox, the least susceptible individuals will survive, and may be the parents of children who will be likely to inherit the special characters as regards structure or physiological activity of organs or tissue elements upon which this comparative immunity depends. The tendency of continuous or repeated exposure to the same pathogenic agent will evidently be to establish a race tolerance; and there is reason to believe that such has been the effect in the case of some of the infectious diseases of man—*e.g.*, syphilis, small-pox—which have been noticed to prevail with especial severity when first introduced among a virgin population, as in the islands of the Pacific, etc.

In the same way we may explain the immunity which carnivorous animals have for anthrax and various forms of septicæmia to which the herbivora are very susceptible when the pathogenic germ is introduced into their bodies by inoculation. From time immemorial the carnivora have been in the habit of fighting over the dead bodies of herbivorous animals, some of which may have fallen a prey to these infectious germ diseases, and in their fighting they receive wounds, inoculated with the infectious material from these bodies, which would be fatal to a susceptible animal. If at any time in the past a similar susceptibility existed among the carnivora, with individual differences as to resisting power, it is evident that there would be a constant tendency for the most sus-

ceptible individuals to perish and for the least susceptible to survive.

The essential difference between a susceptible and immune animal depends upon the fact that in one the pathogenic germ, when introduced by accident or experimental inoculation, multiplies and invades the tissues or the blood, where, by reason of its nutritive requirements and toxic products, it produces changes in the tissues and fluids of the body inconsistent with the vital requirements of the infected animal; while in the immune animal multiplication does not occur, or is restricted to a local invasion of limited extent, and in which, after a time, the resources of nature suffice to destroy the parasitic invader.

But these "resources of nature," upon which natural immunity depends, are not at all times available for the prevention of infection, and may be neutralized by various agencies which demand consideration.

It has been shown by experiment that naturally immune animals may be infected by the addition of certain substances to cultures of pathogenic bacteria. Thus Arloing was able to induce symptomatic anthrax in animals naturally immune for this disease by mixing with his cultures various chemical substances, such as carbolic acid, pyrogallic acid, and especially lactic acid (twenty per cent.). Leo has shown that white mice, which are not subject to the pathogenic action of the glanders bacillus, may be rendered susceptible by feeding them for some time upon phloridzin, which gives rise to an artificial diabetes, and causes the tissues to become impregnated with sugar. Behring claims to have demonstrated by experiment that white rats lose their immunity for anthrax when fed for some time upon

an exclusively vegetable diet, or when phosphate of lime is added to their food, and he has suggested that the immunity of these animals may be due to the highly alkaline reaction of their blood and tissue juices.

Bouchard has shown that very small doses of a pure culture of Bacillus pyocyaneus are fatal to rabbits when at the same time a considerable quantity of a filtered culture of the same bacillus is injected into a vein. The animal could have withstood the filtered culture alone, or the bacillus injected beneath its skin; but its resisting power—natural immunity—is overcome by the combined action of the living bacilli and the toxic substances contained in the filtered culture. The same result may be obtained by injecting sterilized cultures of a different microörganism. Thus Roger has shown that the rabbit, which has a natural immunity against symptomatic anthrax, succumbs to infection when inoculated with a culture of the bacillus of this disease, if at the same time it receives an injection of a sterilized or non-sterilized culture of Bacillus prodigiosus. Monti has succeeded in killing animals with old and attenuated cultures of Streptococcus pyogenes, or of Staphylococcus pyogenes aureus, by injecting at the same time a culture of Proteus vulgaris. In a similar way, it seems probable, the normal resistance of man to infection by certain pathogenic bacteria may be overcome. Thus when water contaminated by the presence of the typhoid bacillus is used for drinking by the residents of a certain town or district, not all of those who in this way are exposed to infection contract typhoid fever, and among those who do, there is good reason to believe that, in certain cases at least, the result depends upon an additional factor of the kind suggested by the above-mentioned experiments,

e.g., the consumption of food containing putrefactive products produced by Proteus vulgaris and other saprophytes, or the respiration of an atmosphere containing the volatile products of putrefaction (*e.g.*, sewer-gas). Recent experiments (1894) made by Alessi, in the Hygienic Institute of the University of Rome, give support to this view. The experiments were made upon rats, guinea-pigs, and rabbits. The rats were confined in a close cage with perforated bottom, which was placed over the opening of a privy; the guinea-pigs and rabbits in similar cages having a receptacle below in which their own excreta was allowed to accumulate. The animals which breathed an atmosphere vitiated in this way lost, after a time, their usual activity and became emaciated, although they continued to eat greedily. When these animals were inoculated with a small quantity of a culture of the typhoid bacillus (0.25 to 0.5 c.c.), they died within from twelve to thirty-six hours. The same amount of the typhoid culture injected into control animals produced no injurious effect. In the animals which succumbed to typhoid infection there was found a hemorrhagic enteritis, increase in volume of Peyer's glands and of the spleen, and typhoid bacilli in the blood, liver, and spleen. The characteristic appearances of typhoid infection were more pronounced in the rabbits and guinea-pigs than in rats. Similar experiments with Bacillus coli communis gave similar results. The time required to induce this predisposition for typhoid infection was from five to seventy-two days for the rats, seven to fifty-eight for the guinea-pigs, and three to eighteen for the rabbits. Alessi found that the susceptibility to infection diminished after a certain time, and suggests that in a similar way man may become habituated to breathing an

atmosphere containing sewer-gases, which for those unaccustomed to it would be likely to induce typhoid infection in case the typhoid bacillus should be introduced into the intestine. An attempt to determine what particular gas was concerned in the neutralization of the natural immunity of the animals experimented upon was not successful. The following gases, tested separately, gave a negative result: Ammonia, hydrogen sulphide, carbon dioxide, carbonic oxide, and ammonium sulphide. The natural immunity of healthy animals may also be neutralized by other agencies which have a depressing effect upon the vital resisting power. Thus Nocard and Roux found by experiment that an attenuated culture of the anthrax bacillus, which was not fatal to guinea-pigs, killed these animals when injected into the muscles of the thigh after they had been bruised by mechanical violence. Abarrin and Roger found that white rats, which are not susceptible to anthrax, became infected and frequently died if they were exhausted, previous to inoculation, by being compelled to turn a revolving wheel for a considerable time. Pasteur found that fowls, which have a natural immunity against anthrax, become infected and perish if they are subjected to artificial refrigeration after inoculation. This has been confirmed by the more recent experiments of Wagner (1891). According to Canalis and Morpurgo, pigeons which are enfeebled by inanition easily contract anthrax as a result of inoculation. Arloing states that sheep which have been freely bled contract anthrax more easily than others; and Serafini found that when dogs were freely bled the bacillus of Friedländer, injected into the trachea or the pleural cavity, entered, and apparently multiplied to some extent in the blood, whereas without such pre-

vious bleeding they were not to be found in the circulating fluid. Certain anæsthetic agents have also been shown to produce a similar result. Platania communicated anthrax to immune animals—dogs, frogs, pigeons—by bringing them under the influence of curare, chloral, or alcohol; and Wagner obtained similar results in his experiments upon pigeons to which he had administered chloral. In man, clinical experience shows that those who are addicted to the excessive use of alcohol are especially liable to contract certain infectious diseases—pneumonia, erysipelas, yellow fever, etc.

The micrococcus of pneumonia is habitually present in the salivary secretions of many healthy individuals, and it is evident that an attack of pneumonia does not depend alone upon the presence of this micrococcus, which has, nevertheless, been conclusively shown to be the usual infectious agent in cases of croupous pneumonia. No doubt the introduction of the pathogenic micrococcus to the vulnerable point—the lungs—is an essential factor in the development of a case of pneumonia, but there is reason to believe that there are other factors equally essential. Thus it is well known that an attack of pneumonia often results from exposure to cold, which may act as an exciting cause; and, also, that a recent attack of an acute febrile disease—especially measles—constitutes a predisposing cause. It is generally recognized that mal-nutrition, want of exercise, insanitary surroundings, and continued respiration of an atmosphere loaded with dust, as in cotton-mills, or a recent attack of pneumonia, constitute predisposing causes to tubercular infection by way of the lungs.

While natural immunity may be overcome by the various depressing agencies referred to, it is also true that it

has only a relative value in the absence of these predisposing causes, and may be overcome by unusual virulence of the pathogenic infectious agent, or by the introduction into the body of an excessive amount of a pure culture of the same.

The pathogenic potency of known disease germs varies as widely as does the susceptibility of individuals to their specific action. In general it may be said that the more recently the germ comes from a developed case of the disease to which it gives rise, the more virulent it is, and the longer it has been cultivated outside of the animal body the more attenuated is its pathogenic power. Thus when the discharges of a typhoid-fever patient find their way directly to a water-supply of limited amount a large proportion of those who drink the water are likely to be attacked; but when a considerable interval of time has elapsed since the contamination occurred, although the germs may still be present, the liability to attack is much less on account of diminished pathogenic virulence.

The development of an attack also depends, to some extent, upon the number of germs introduced into a susceptible individual at one time. The resources of nature may be sufficient to dispose of a few bacilli, while a large number may overwhelm the resisting power of the individual.

The experiments of Cheyne (1886) show that in the case of very pathogenic species, a single bacillus, or at least a very small number, introduced beneath the skin, may produce fatal infection in a very susceptible animal, while greater numbers are required in those less susceptible. Thus a guinea-pig succumbed to general infection after being inoculated subcutaneously with anthrax

blood diluted to such an extent that, by estimation, only one bacillus was present in the fluid injected; and a similar result was obtained in mice with Bacillus murisepticus. In the case of the microbe of fowl cholera (Bacillus septicæmia hemorrhagicæ), Cheyne found that for rabbits the fatal dose was 300,000 or more, that from 10,000 to 300,000 cause a local abscess, and that less than 10,000 produce no appreciable effect. The common saprophyte, Proteus vulgaris, was found to be pathogenic for rabbits when injected into the dorsal muscles in sufficient numbers. But, according to the estimates made, 225,000,000 were required to cause death, while doses of from 9,000,000 to 112,000,000 produced a local abscess, and less than 9,000,000 gave an entirely negative result.

In the scale of living things man stands at the head, and the unicellular organisms known as bacteria at the foot, yet the relations of these "microbes" to the "lord of creation" are more important and more complex than those which exist between man and any other single group of living organisms.

In the absence of bacteria, or of some other organisms to perform their functions, the continued existence of man upon the face of the globe would be impossible; for it is due to their activity in the decomposition of animal and vegetable substances that organic material, stored up as a result of the vital activities of higher plants and animals, is returned to the soil and atmosphere after the death of these. Without such a provision of nature organic life would long since have come to a standstill, from the storing up of those essential elements which go to make up the structure of animal and vegetable tissues. But as a result of the decomposition of such tissues after death, through the agency of bacteria, these elements

are released from the complex combinations in which they exist and again become available for the nutrition of living plants and animals. The moment life is extinct, when temperature conditions are favorable, these destructive processes commence. The saprophytic bacteria, constantly present in the alimentary canal, quickly invade the tissues, and soon the whole body is invaded by these agents of putrefactive decomposition. But the living body not only resists the invasion of these organisms through the walls of the intestine and the external integument, but is able to dispose of a considerable number of putrefactive microörganisms when these are injected into the circulation. Now, to some extent at least, the resistance to invasion by pathogenic bacteria—natural immunity—corresponds with the resistance to the putrefactive organisms referred to, and no doubt depends upon similar causes. Some of these pathogenic bacteria are very commonly found upon the surface of the body of healthy individuals or upon exposed mucous membranes. As already mentioned, the micrococcus of croupous pneumonia is very frequently found in the salivary secretions of healthy persons, and it seems probable that its normal habitat is the human mouth.* The pus cocci —Staphylococcus aureus and albus—are not infrequently found in the mouths or on the surface of the body of healthy persons.† And during the prevalence of diphthe-

* Netter found it in the salivary secretions of fifteen per cent. of the healthy individuals examined—one hundred and sixty-five in all.

† Netter found Staphylococcus aureus seven times in the salivary secretions, out of one hundred and twenty-seven individuals examined. Steffeck (1892) examined the vaginal secretions of twenty-nine pregnant females, and found S. pyogenes albus in nine, S. pyogenes aureus in three, and Streptococcus pyogenes in one. In nasal mucus Von Besser, in eighty-one cases examined, found "diplococcus pneumoniæ" fourteen times, S. pyogenes aureus fourteen times, and Streptococcus pyogenes seven times.

ria in a household, diphtheria bacilli have been found in the mouths of individuals who presented no evidence of diphtheria infection. When the normal resisting power of the tissues is overcome locally by mechanical violence, by arrest of circulation, by freezing, etc., the ordinary putrefactive bacteria may invade the injured tissues, and as a result we have gangrene. Or an invasion by pus cocci may give rise to a localized inflammation, resulting in abscess formation. Again, certain conditions relating to the diet and surroundings of an individual may constitute a predisposition to local infectious processes of greater or less extent, due to invasion of the tissues by the common pathogenic micrococci above referred to. Thus we have developed acne pustules, boils, carbuncles, etc.

Explanation of Natural Immunity.

We have now to inquire upon what the natural immunity depends which enables the healthy animal body to resist invasion by the destructive agents referred to.

Phagocytosis.—In my chapter on "Bacteria in Infectious Diseases," in "Bacteria," published in the spring of 1884, but placed in the hands of the publishers in 1883, I say:

"It may be that the true explanation of the immunity afforded by a mild attack of an infectious germ disease is to be found in an acquired tolerance to the action of a chemical poison produced by the micro-organism, and consequent ability to bring the resources of nature to bear to restrict invasion by the parasite."

In the same chapter the resources of nature supposed to be brought to bear in restricting invasion by the parasite are referred to as follows:

"If we add a small quantity of culture-fluid containing the bacteria of putrefaction to the blood of an animal withdrawn from the circulation into a proper receptacle and maintained in a culture-oven at blood-heat, we will find that these bacteria multiply abundantly, and evidence of putrefactive decomposition will soon be perceived. But if we inject a like quantity of the culture-fluid, with its contained bacteria, into the circulation of a living animal, not only does no increase and no putrefactive change occur, but the bacteria introduced quickly disappear, and at the end of an hour or two the most careful microscopical examination will not reveal the presence of a single bacterium. This difference we ascribe to the vital properties of the fluid as contained in the vessels of a living animal, and it seems probable that the little masses of protoplasm known as white blood-corpuscles are the essential histological elements of the blood, as far as any manifestation of vitality is concerned. The writer has elsewhere (1881) *suggested that the disappearance of the bacteria from the circulation, in the experiment referred to, may be effected by the white corpuscles*, which, it is well known, pick up, after the manner of amœbæ, any particles, organic or inorganic, which come in their way. And it requires no great stretch of credulity to believe *that they may, like an amœba, digest and assimilate the protoplasm of the captured bacterium, thus putting an end to the possibility of its doing any harm.*

"In the case of a pathogenic organism we may imagine that, when captured in this way, it may share a like fate if the captor is not paralyzed by some potent poison evolved by it, or overwhelmed by its superior vigor and rapid multiplication. In the latter event the active career of our conservative white corpuscles would be quickly terminated and its protoplasm would serve as food for the enemy. It is evident that in a contest of this kind the balance of power would depend upon circumstances relating to the *inherited* vital characteristics of the invading parasite and of the invaded leucocyte."

This explanation is now very commonly spoken of as the "Metschnikoff theory," although, as a matter of fact, it was clearly stated by the writer several years (1881) before Metschnikoff's first paper (1884) was published. Metschnikoff has, however, been the principal defender of this explanation of acquired immunity, and has made extensive and painstaking researches, as a result of which many facts have been brought to light which appear to give support to the present writer's hypothesis—the so-called Metschnikoff theory.

The observations which first led Metschnikoff to adopt the explanation of immunity under consideration were made upon a species of daphnia, which is subject to infection by a torula resembling the yeast fungus. Entering with the food, this fungus penetrates the walls of the intestine and invades the tissues. In certain cases the infection does not prove fatal, owing, as Metschnikoff asserts, to the fact that the fungus cells are seized upon by the leucocytes, which appear to accumulate around the invading parasite (chemiotaxis) for this special purpose. If they are successful in overpowering and destroying the parasite, the animal recovers; if not, it succumbs to the general infection which results. In a similar manner, Metschnikoff supposes, pathogenic bacteria are destroyed when introduced into the body of an immune animal. The colorless blood-corpuscles which he denominates phagocytes, accumulate at the point of invasion and pick up the bacteria, as they are known to pick up inorganic particles injected into the circulation. So far there can be no doubt that Metschnikoff is right. The presence of bacteria in the leucocytes in considerable numbers, both at the point of inoculation and in the general circulation, has been repeatedly demonstrated

in animals inoculated with various pathogenic bacteria. The writer observed this in his experiments, made in 1881, in which rabbits were inoculated with cultures of his "Micrococcus Pasteuri" (micrococcus of croupous pneumonia), and it was this observation which led him to suggest the hypothesis which has since been so vigorously supported by Metschnikoff. But the presence of a certain number of bacteria within the leucocytes does not prove the destructive power of these cells for living pathogenic organisms. As urged by Weigert, Baumgarten, and others, it may be that the bacteria were already dead when they were picked up, having been destroyed by some agency outside the blood-cells; and, as we shall see later, there is experimental evidence that blood-serum has decided germicidal power for certain pathogenic bacteria, and that the blood-serum of the rat and other animals, which have a natural immunity against anthrax, is especially fatal to the anthrax bacillus. On the other hand, there is reason to believe that living bacteria picked up by leucocytes are not always destroyed, but that, in some instances at least, they thrive and multiply within these protoplasmic masses. In mouse septicæmia and in gonorrhœa one would be disposed to decide, from the appearance and arrangement of the pathogenic bacteria in the leucocytes, that they are not destroyed but that they multiply in the interior of these cells, which in the end succumb to this parasitic invasion. In both of the diseases mentioned we find the leucocytes so completely filled with the pathogenic microorganisms that it is difficult to believe that they have all been picked up by a voracious phagocyte, which has stuffed itself to repletion, while numerous other leucocytes in the same microscopic field have failed to capture

a single bacillus or micrococcus. Moreover, the staining of the parasitic invaders, and the characteristic arrangement of the "gonococcus" in stained preparations of gonorrhœal pus, indicate that their vitality has not been destroyed in the interior of the leucocytes or pus-cells, and we can scarcely doubt that the large number found in certain cells is due to multiplication *in situ* rather than to an unusual activity of these particular cells. But in certain infectious diseases, and especially in inoculations of the anthrax bacillus into immune animals, the bacilli included within the leucocytes often give evidence of degenerative changes, which would support the view that they are destroyed within the leucocytes, unless these changes occurred before they were picked up, as claimed by Nuttall and others.

Metschnikoff divides the so-called phagocytes into two groups: Fixed phagocytes (endothelial cells, etc.) and free phagocytes. According to his observations not all leucocytes are phagocytes. The comparatively small, immobile leucocytes ("lymphocytes") with a single large nucleus never take up bacteria. The large uninuclear leucocyte which exhibits active amœboid movements, and in which the nucleus is frequently lobed or reniform, is called by Metschnikoff a macrophage, and the smaller multinuclear leucocyte (or with one nucleus in process of breaking up) he calls a microphage. The macrophages are believed to be largely of endothelial origin. Metschnikoff asserts that in general the more virulent the microörganism the rarer is its presence observed within the phagocytes. Thus in the acute septicæmias which are quickly fatal to susceptible animals—*e.g.*, fowl cholera, rabbit septicæmia, anthrax in mice, etc.—the pathogenic bacteria are rarely found in the inte-

rior of the cells. They remain free in the vicinity of the point of inoculation and thence quickly invade the blood. On the other hand, in infectious diseases having a more protracted course there is a very decided phagocytosis. As an example of this Metschnikoff mentions mouse septicæmia " which has a duration in the mouse two and a half times as long as that of anthrax in the same animal," and the chronic infectious diseases—tuberculosis, leprosy, rhinoscleroma, and glanders. Again, attention is called to the phenomena attending recovery from certain infectious diseases. In relapsing fever, for example, during the sudden access of the fever the spirilla are present in the blood in great numbers, and they are not included within the cells. During the apyretic stage they disappear from the blood, but are found included in the phagocytes of the spleen. In like manner rats and pigeons which survive an attack of anthrax, when killed during the period of resolution show numerous bacilli included within leucocytes and splenic phagocytes, and very few free bacilli.

In a similar manner, according to Metschnikoff, animals which have an acquired immunity for anthrax or other infectious diseases of bacterial origin, when inoculated subcutaneously, resist infection because there is an extensive emigration of leucocytes to the point of inoculation, and these soon include the bacteria in large numbers. In susceptible animals, not having thus acquired immunity, the phagocytes are inoperative, and the bacteria are very rarely seen included within them. As examples of this Metschnikoff cites the bacillus of anthrax and Vibrio Metschnikovi. In unvaccinated rabbits inoculated with anthrax, phagocytosis is said to be very seldom seen, while in rabbits protected by " vaccination "

it is very marked. The difference is still more remarkable in guinea-pigs inoculated with Vibrio Metschnikovi. In vaccinated animals the phagocytes are loaded with the microbes, in those not vaccinated they are not seen included in these cells. According to Metschnikoff the giant cells of tuberculosis are "huge multinuclear phagocytes" in which the bacilli are destroyed. This is shown by the "very evident signs of degeneration; the bacilli swell, their enveloping membrane becomes much thickened and highly refractive, and in time the contents lose the power of fixing the staining material, so that, eventually, nothing is left but slightly yellowish forms, recalling, in proportions and position, the enlarged bacilli."

Action of Blood-Serum and Other Organic Liquids Upon Bacteria.—Bacteriologists have long been aware of the fact that many species of bacteria, when injected into the circulation of a living animal, soon disappear from the blood, and that the blood of such an animal a few hours after the intravenous injection, of putrefactive bacteria for example, does not contain living bacteria capable of development in a suitable culture medium. Wyssokowitsch, in an extended series of experiments, has shown that non-pathogenic bacteria may be obtained in cultures from the liver, spleen, kidneys, and bone marrow after they have disappeared from the blood; but that, as a rule, those present in these organs have lost their vitality, as shown by culture experiments, in a period varying from a few hours to two or three days. As has been stated above, this disappearance was accounted for by the writer (1881), and later by Metschnikoff (1884), as the result of the vital activities of the leucocytes; but more recently experimental evidence has been presented which

indicates that this is not the only, and probably not the principal, agency by which bacteria introduced into the circulation of living animals are destroyed.

Von Fodor (1887) first called attention to the fact that anthrax bacilli may be destroyed by freshly drawn blood; and Nuttall (1888), in an extended series of experiments, showed that various bacteria are destroyed within a short time by the fresh blood of warm-blooded animals. Thus the anthrax bacillus in rabbit's blood was usually killed in from two to four hours when the temperature was maintained at 37° to 38° C., and the same result was obtained with pigeon's blood at 41° C. But when the blood was allowed to stand for some time, or was subjected to a temperature of 55° C., it no longer possessed germicidal properties and served as a culture-fluid in which an abundant development of anthrax bacilli occurred. Bacillus subtilis and Bacillus megatherium were also destroyed by fresh rabbit's blood, but it was without action upon Staphylococcus pyogenes aureus, which, at a temperature of 37.5° C., was found to have increased in numbers at the end of two hours. Further researches by Behring and Nissen (1890) show that there is a wide difference in the blood of different animals as to germicidal power, and that certain bacteria are promptly destroyed, while other species are simply restrained for a time in their development or are not affected. Thus Nissen found that the cholera spirillum, the bacillus of anthrax, the bacillus of typhoid fever, and Friedländer's bacillus were killed, while Staphylococcus pyogenes albus and aureus, Streptococcus pyogenes, the bacillus of fowl cholera (Bacillus septicæmiæ hæmorrhagicæ), the bacillus of rothlauf and Proteus hominis were able to multiply in rabbit's blood after having been restrained for

a short time in their development. In the case of the cholera spirillum a period of ten to forty minutes sufficed for the complete destruction of a limited number, but when the number exceeded 1,200,000 per c.c. they were no longer destroyed with certainty, and after five hours an increase occurred. The anthrax bacillus was commonly destroyed within twenty minutes, and the typhoid bacillus at the end of two hours. In the experiments of Behring and Nissen it was found that the most pronounced germicidal effect was obtained from the blood of the rat, an animal which has a natural immunity against anthrax; while the blood of the guinea-pig, a very susceptible animal, had no restraining effect and served as a favorable culture-medium for the anthrax bacillus. And the remarkable fact was developed that when the blood of the rat was added to the blood of the guinea-pig, in the proportion of 1 to 8, it exercised a decided restraining influence on the growth of the anthrax bacillus. Later researches have shown that cultivation in the blood of an immune animal causes an attenuation of the virulence of an anthrax culture (Ogata and Jasuhara); also that the injection of the blood of a frog or of a rat into a susceptible animal which has been inoculated with a virulent culture of the anthrax bacillus will restrain the development of the pathogenic bacillus and prevent the death of the inoculated animal.

Buchner (1889) first proved by experiment that the germicidal power of the blood of dogs and rabbits does not depend upon the presence of the cellular elements, but is present in clear serum which has been allowed to separate from the clot in a cool place. Exposure for an hour to a temperature of 55° C. destroys the germicidal action of serum as well as of blood; the same effect is

produced by heating to 52° C. for six hours, or to 45.6° C. for twenty hours. The germicidal power of blood-serum is not destroyed by freezing and thawing, but is lost after it has been kept for some time at ordinary temperatures.

The researches of Buchner, of Hankin, and others, show that this germicidal power of fresh blood-serum depends upon the presence of proteids, to which the first-named bacteriologist has given the name of "alexins." Hankin, in his paper upon the origin of these "defensive proteids" in the animal body (1892), arrives at the conclusion that while they are present in the cell-free serum they are the product of certain leucocytes—Ehrlich's eosinophil cells. He believes that the eosinophil granules become dissolved in the serum and constitute the germicidal proteid which is shown to be present by experiments upon bacteria. According to Hankin the separation of these granules can be witnessed under the microscope. They first accumulate upon one side of the cell and then gradually disappear, and as this occurs a considerable increase in the bactericidal power of the serum can be demonstrated. The germicidal power of the blood-serum is also said to be increased when the number of leucocytes is considerably augmented, as occurs when a sterilized culture of Vibrio Metschnikovi is injected subcutaneously. Also by treatment which favors a separation of the alexin from the leucocytes, *i.e.*, a solution of the eosinophil granules. This may be accomplished by the injection of an extract of the thymus gland of the calf, or by simply allowing the drawn blood to stand for several hours at a temperature of 38° to 40° C.

Buchner's latest communication upon the subject shows that he also attributes the origin of the germicidal

proteid in fresh blood-serum to the leucocytes. In his paper on "Immunity," read at the Eighth International Congress on Hygiene and Demography (Budapest, 1894) he calls attention in the first place to the fact that a clearly marked distinction must be made between natural immunity and acquired immunity, inasmuch as the "alexins" and "antitoxins" have very different properties. The first-mentioned proteids are destroyed by a comparatively low temperature (55° to 60° C.) while the antitoxins resist a considerably higher temperature, and, unlike the alexins, have no bactericidal or globulicidal action. A very remarkable fact developed in Buchner's experiments is that the blood-serum from the dog and from the rabbit, when mixed, neutralize each other so far as their germicidal power is concerned.

By injecting sterilized emulsions of wheat-flour paste in the pleural cavity of rabbits and dogs Buchner succeeded in obtaining an exudate which had more decided germicidal power than the blood or serum of the same animal. This was evidently due to the large number of leucocytes present, but not to their phagocytic action, as was shown by experiment. By freezing the exudate the leucocytes were killed, but the germicidal action of the fluid was rather increased than diminished by freezing. While freezing had no effect upon the germicidal action of the pleural exudate this was always neutralized by exposure to a temperature of 55° C. The observations referred to lead Buchner to the conclusion, which we concur in, that phagocytosis plays an entirely subordinate *rôle* in the germicidal action of freshly drawn blood, but that this action does depend, to a considerable extent at least, upon the leucocytes, inasmuch as the soluble proteid to which it is due has its origin from these proto-

plasmic masses. Buchner says in his recent paper, above referred to:

"Upon the basis of the results reached a reconciliation with the theory of phagocytosis seems very possible, as the researches upon the bactericidal action of blood and serum lead to the final conclusion that the leucocytes are the bearers of the bactericidal material. The extended facts of observation which Metschnikoff and his associates have collected in favor of phagocytic action remain undisturbed. But the explanation of the process, the conception of the causal connection, must be somewhat different. Through such a conception the observations opposed to the theory of phagocytosis will also find their explanation. Thus in the observations of Ribbert, which led to the statement of his wall-forming theory, the 'mantel' of leucocytes which surrounds the infected focus may very well represent a protecting wall which acts, not by phagocytosis but through the germicidal proteid given off by the leucocytes. Also in streptococcus infection recovery often occurs without any evidence of phagocytosis. Müller, in recent experiments on anthrax in rats, has failed entirely to observe phagocytosis, although the organism of the rat is very unfavorable for anthrax infection. Also in relapsing fever Tictin was not able to discover any phagocytosis in apes from which the spleen had been removed, either during the attack or after recovery, when this occurred. And R. Pfeiffer recently demonstrated, in his interesting researches upon the killing of cholera vibrios in the peritoneal cavity of immunized guinea-pigs, that their rapid destruction occurred without phagocytosis. All of these cases present no difficulty as soon as the idea is abandoned that the act of devouring ('Auffressen') constitutes a *conditio sine qua non* for the bactericidal activity of the leucocytes."

Emmerich, Tsuboi, Steinmetz, and Löw (1892), as a result of extended experiments, arrived at the conclusion that the germicidal action of blood-serum "depends

upon a specific property of the alkali-serum-albumin, and that it is a purely chemical process." They state that when the germicidal power is neutralized by heat it may be restored by the addition of an alkali. Buchner repeated the experiments of Emmerich and his associates and obtained similar results, but interprets them differently. According to him the serum does not regain its germicidal power, but after the addition of an alkali and subsequent dialyzing the nutritive value of the serum is so diminished that the bacteria do not develop in it.

The failure of the anthrax bacillus to develop in inoculated white rats has been ascribed by Behring (1888) to the highly alkaline reaction of the blood and tissue juices of this animal. Hankin (1891) in extended experiments arrived at a different conclusion. From the spleen and blood-serum of rats he isolated a globulin possessing germicidal properties, to which he ascribes the power of the rat's blood to destroy anthrax bacilli, without, however, rejecting the view that the excessive alkalinity of the blood of this animal may be a factor in producing this result. Pane (1892) has made experiments which give additional weight to the assumption that the alkalinity of the blood is an important factor in accounting for immunity. He states that carbonate of soda, dissolved in water, in the proportion of 1 to 3,000, has a decided germicidal action upon the anthrax bacillus, equal to that of the blood-serum of the rabbit. And that when rabbit serum is completely neutralized it no longer has any injurious action on anthrax bacilli.

Zagari and Innocente (1892) also arrived at the conclusion that the diminished resistance to anthrax infection resulting from curare-poisoning in frogs, and from chloral or alcohol in dogs (Platania), in fowls as a result

of starvation (Canalis and Morpurgo), in white mice as a result of fatigue (Charin and Roger), is, in fact, due to diminished alkalinity of the blood, which they found to correspond with the increased susceptibility resulting from the causes mentioned.

Buchner (1892) states that several of the ammonium salts, and especially ammonium sulphate, cause an increase in the germicidal action of blood-serum, and also increase its resistance to the neutralizing effects of heat. The experiments of Pansini and Calabrese (1894) show, on the contrary, that the addition of uric acid to blood-serum diminishes its bactericidal activity, as does also the presence of glucose. That certain infectious diseases are especially virulent in persons suffering from diabetes is a frequently repeated clinical observation.

Van Fodor has shown by experiment that the injection of an alkali into the circulation of a rabbit increases its resistance to anthrax infection and the germicidal activity of its blood-serum. The same bacteriologist has found that when a rabbit is infected with anthrax, the alkalinity of its blood is notably increased during the first twenty-four hours, when we may suppose that the powers of nature are brought to bear to resist the invading parasite, and that after this time it rapidly diminishes. Ten hours after infection (by subcutaneous inoculation?) the alkalinity of the blood had increased 21.5 per cent. Shortly before the death of the animal a diminution of 26.3 per cent. was noted. This diminution was observed in thirty-four out of thirty-nine animals experimented upon, and these animals succumbed to the anthrax infection in a shorter time than did the other five in which there was no such diminution.

It seems probable that the germicidal property of

freshly drawn blood-serum is not due to its alkalinity, *per se*, but to the fact that the germicidal constituent is only soluble in an alkaline fluid. The recent researches of Vaughn, McClintock, and Novy indicate that this germicidal constituent is a nuclein. Dr. Vaughn in his last published paper upon "Nucleins and Nuclein Therapy," says: "Kossel, of Berlin, has confirmed our statements concerning the germicidal action of the nucleins. Dr. McClintock and I have also demonstrated that the germicidal constituent of blood-serum is a nuclein. This nuclein is undoubtedly furnished by the polynuclear white corpuscles." Denys has recently (1894) reported the results of experiments made in his laboratory by Van der Velde, which give support to the conclusion reached by Vaughn. In these experiments a sterilized culture of staphylococci was injected into the pleural cavity of rabbits in order to obtain an exudate. At intervals of two hours this exudate was obtained by killing one of the animals in the series experimented upon, and at the same time blood from the animal was secured. Both the exudate and the blood was placed in a centrifugal machine, in order to obtain a serum free from corpuscular elements. The germicidal activity of the serum was then tested. The general result of the experiments was to show that the longer the interval after the injection into the pleural cavity, the more potent the germicidal activity of the exudate became; and that there was no corresponding increase in the activity of the blood-serum obtained from the circulation. At the end of ten or twelve hours, the serum from the exudate killed all of the staphylococci in a bouillon culture twenty times as great in quantity as the germicidal serum used in the experiment. The absence of any increase in germicidal

power in the blood-serum taken from the general circulation shows that the notable increase manifested by the exudate was due to local causes; and as a matter of fact it corresponded with an increase in the number of leucocytes as found in the pleural exudate.

Thus it will be seen that the independent researches of Hankin, of Buchner, of Vaughn, and of other competent bacteriologists, have led them to the same ultimate result so far as the origin of the germicidal constituent of the blood is concerned, and that the leucocytes appear to play an important *rôle* in the protection of the animal body from invasion by bacteria (natural immunity), although the method by which this is accomplished differs from that suggested by the writer in 1881, and since strongly supported by Metschnikoff and his associates—"phagocytosis."

With reference to the physiological and chemical characters of the nucleins I quote from Vaughn's paper, above referred to, as follows:

"Physiologically nucleins may be said to form the chief chemical constituent, of the living parts of cells. Speaking broadly, we may say that the nuclein is that constituent of the cell by virtue of which this histologic unit grows, develops, and reproduces itself. It is the function of the nuclein of the cell to utilize the pabulum within its reach. It must be evident that those tissues most abounding in cellular elements contain relatively the largest amounts of nuclein. It must also be seen that it is by virtue of their nuclein that the cells of various organs and organisms possess and manifest their individual peculiarities. We should therefore expect to find that the nuclein of the yeast-cell is not identical with that of the bacillus tuberculosis, and that the nuclein of the spleen differs from that of the thyroid gland. The number of kinds of nuclein is limited only by the varie-

ties of cells. Nuclein is the chemical basis of that part of the cell designated by the histologist as the nucleus, sometimes called chromatin on account of the readiness with which it absorbs and holds coloring agents. It is the nuclein of the bacterium which takes up and retains the stains, and it is on account of the fact that the nuclein of the bacillus tuberculosis differs from that of other bacilli that we are able to distinguish the former from the latter by its tinctorial properties. Differences in reaction with staining reagents, so plainly seen under the microscope, are only outward manifestations of less apparent and more important differences in chemical composition.

"Chemically the nucleins are complex, proteid bodies, characterized especially by the large amount of phosphorus which they contain. The phosphorus exists in the form of nucleinic acid, which is combined with a highly complex basic substance. So far as we know at present, the nucleinic acid of all nucleins is the same, yet the basic part differs in the various nucleins. This basic substance yields, as decomposition products, one or more of the so-called xanthin bodies; adenin, guanin, sarkin, and xanthin. Some nucleins yield only adenin, and these may be designated as adenyl nucleinic acids. Those which furnish xanthin most abundantly may be called xanthyl nucleinic acids. *Generally speaking, the nucleins are insoluble in dilute acids and soluble in dilute alkalies.* They resist peptic digestion and in this way may be separated from most other proteid bodies."

It has been shown by several investigators that the number of leucocytes increases in certain infectious diseases, and this increase, together with an increased alkalinity of the blood, which has heretofore been referred to, appears to be a provision of nature for overcoming the infection which has already occurred. Billings,* in a re-

* J. S. Billings, Jr.: The Leucocytes in Croupous Pneumonia, Bulletin of the Johns Hopkins Hospital, November, 1894, p. 105.

cent paper, gives the evidence as regards croupous pneumonia. Some of his conclusions are given below :

"In cases of pneumonia pursuing a favorable course there is, as a rule, a marked increase in the number of leucocytes during the febrile period of the disease. This leucocytosis is probably present at the time of the chill, and may be very marked within a few hours."

"In cases showing extensive involvement of both lungs the leucocytes are apt to reach a higher point than in those cases where the involvement is only moderate."

"The fatal cases may show either the presence or absence of leucocytosis. In those cases showing a leucocytosis, some other cause of death than the virulence of the bacterial poison must be sought for."

"The prognosis in cases showing a complete and continuous absence of leucocytosis is unfavorable as a rule."

"The leucocytosis in pneumonia is a so-called pure leucocytosis, *i.e.*, an increase in the polynuclear elements solely."

The experimental evidence submitted, considered in connection with the extensive literature relating to "phagocytosis," leads us to the conclusion that natural immunity is due to a germicidal substance present in the blood-serum which has its origin (chiefly, at least) in the leucocytes and is soluble only in an alkaline medium. And that local infection is usually resisted by an afflux of leucocytes to the point of invasion ; but that phagocytosis is a factor of secondary importance in resisting parasitic invasion. Also that general infection, at least in some infectious diseases, is resisted, and in non-fatal cases overcome, by an increase in the number of leucocytes and in the alkalinity of the blood-serum—which favors solution of the germicidal proteids contained in the polynuclear leucocytes.

This conclusion is in accord with certain other facts which may be briefly referred to. Numerous experiments show that natural immunity may be overcome by infection with an excessive number of pathogenic bacteria and their products, as contained in a culture; or by an unusually virulent variety. According to Denys the pathogenic bacteria, in general, resist the germicidal action of rabbit serum better than the saprophytic bacteria; and of two varieties of the same pathogenic species the most virulent has the greater resistance to this germicidal action. This is in accord with the fact that natural immunity protects completely from invasion by the ordinary saprophytes, by which man and the lower animals are surrounded on all sides. It is only when the circulation is arrested in the entire body, or in a portion of it, that the putrefactive bacteria succeed in invading the tissues.

The action of the germicidal substance present in blood-serum upon bacteria appears to be a quantitive one so far as the bacteria are concerned, and it may also be neutralized by their products. Thus Bastin (1892) concludes from his experiments that the germicidal action of blood-serum from the dog is neutralized or greatly diminished by the intravenous injection of cultures of bacteria (S. pyogenes aureus, Bac. lactis ærogenes) in considerable amounts, and that this occurs as well when the bacteria in the cultures have been killed by heat as when they are living. This effect is manifested within two minutes after the injection, and is said to reach its maximum inside of ten minutes; at the same time there is a diminution in the coagulability of the blood. At the end of five or six hours the normal coagulability and germicidal action has been recovered. The

same is true as regards freshly drawn blood, *in vitro*. When bacteria or their products are added in excess the germicidal action is no longer manifested, but multiplication occurs at once, as in a favorable culture-fluid. Thus Nissen found that a limited number of cholera spirilla added to freshly drawn rabbit's blood perished in from ten to forty minutes; but when the number exceeded 1,200,000 per cubic centimetre an increase occurred at the end of five hours.

It has been demonstrated by several experimenters that other albuminous fluids possess a germicidal power similar to that manifested by freshly drawn blood. Thus Nuttall found that a pleuritic exudate from man destroyed the anthrax bacillus in an hour, the aqueous humor of a rabbit in two hours. Prudden found that the albuminous fluid obtained from a hydrocele sac, or from the abdominal cavity in ascites, possesses similar germicidal power, and Fokker has demonstrated that fresh milk destroys the vitality of certain bacteria.

Hankin has extracted from the spleen and lymphatic glands of dogs and cats a "cell globulin" which possesses germicidal power similar to that of blood-serum. This he obtained by treatment with a solution of sodium chloride and subsequent precipitation with alcohol. This "globulin" is insoluble in water or alcohol, and does not dialyze.

II.

ACQUIRED IMMUNITY.

It has long been known that, in a considerable number of infectious diseases, a single attack, however mild, affords protection against subsequent attacks of the same disease; that in some cases this protection appears to be permanent, lasting during the life of the individual; that in others it is more or less temporary, as shown by the occurrence of a subsequent attack.

The protection afforded by a single attack not only differs in different diseases, but in the same disease varies greatly in different individuals. Thus certain individuals have been known to suffer several attacks of small-pox or of scarlet fever, although, as a rule, a single attack is protective. Exceptional susceptibility or insusceptibility may be not only an individual but a family characteristic, or it may belong to a particular race.

In those diseases in which second attacks are not infrequent, as, for example, in pneumonia, in influenza, or in Asiatic cholera, it is difficult to judge from clinical experience whether a first attack exerts any protective influence. But from experiments upon the lower animals we are led to believe that a certain degree of immunity, lasting for a longer or shorter time, is afforded by an attack of pneumonia or of cholera, and probably of all infectious diseases due to bacterial parasites. In the malarial fevers, which are due to a parasite of a different

class, one attack affords no protection, but rather predisposes to a subsequent attack.

In those diseases in which a single attack is generally recognized as being protective exceptional cases occur in which subsequent attacks are developed as a result of unusual susceptibility or exposure under circumstances especially favorable to infection. Maiselis has recently (1894) gone through the literature accessible to him for the purpose of determining the frequency with which second attacks occur in the various diseases below mentioned. The result is as follows:

	Second Attacks.	Third Attacks.	Fourth Attacks.	Total.
Small-pox.....	505	9	0	514
Scarlet fever	29	4	0	33
Measles	36	1	0	37
Typhoid fever.....	202	5	1	208
Cholera	29	3	2	34

These figures support the view generally entertained by physicians that second attacks of scarlet fever and of measles are comparatively rare, while second attacks of small-pox are not infrequently observed. Considering the very large number of cases of typhoid fever which occur annually in all parts of Europe and America the number of second attacks collected does not bear a very large proportion to the total number taken sick, although the recorded cases, of course, fall far short of the total number of second attacks of this and the other diseases mentioned.

The second attacks of cholera recorded are not numerous, and, no doubt, a carefully conducted investigation made in the areas of endemic prevalence of this disease would show that second attacks are more common than is indicated by these figures.

The experimental evidence relating to protective inoculations in infectious diseases dates from the discovery by Jenner (1768) of the protection afforded against small-pox by vaccination with lymph taken from the vesicles of cow-pox.

To Pasteur must be accorded the credit of having first shown by the experimental method that animals may be made immune against other infectious diseases than the one mentioned by inoculations with an "attenuated virus." Commencing with his experiments upon chicken cholera, in 1880, we shall briefly trace the development of our knowledge up to the present date.

Having demonstrated that the disease of fowls known as chicken cholera is due to a specific microörganism, which he was able to cultivate in artificial media, Pasteur discovered that his cultures became "attenuated" as to their pathogenic power when they had been kept for some time in the laboratory, and that fowls inoculated with these attenuated cultures suffered a comparatively mild and non-fatal attack of the disease, and were subsequently immune against the pathogenic action of the most virulent cultures, or against contracting the disease by contact with other fowls suffering from it.

Pasteur at once comprehended the importance of this discovery, and inferred that what was true of one infectious germ-disease was likely to be true of others. Subsequent researches, by this *savant*, and by other bacteriologists, have justified this anticipation; and the experimental demonstration has been made in a considerable number of similar diseases.

Pasteur first obtained an attenuated virus for his protective inoculations against chicken cholera by keeping his cultures for a considerable time freely exposed to the

air, and ascribed the attenuation to the action of atmospheric oxygen. He found that when cultures were made from the blood of fowls which died from a chronic form of the disease, they possessed an exceptional virulence which was not lost when the cultures were renewed at short intervals; but that by keeping these cultures for two months the virulence was greatly diminished, and fowls usually recovered when inoculated with such cultures. When kept still longer, his cultures finally lost all pathogenic power. In subsequent experiments with the bacillus of anthrax, Pasteur found that the spores of this bacillus retain their virulence for years, and that it was necessary to exclude them from cultures which were to serve for protective inoculations. When cultivated at a temperature of 42° to 43° C., this bacillus does not form spores; and Pasteur ascertained that cultures kept at this temperature for eight days no longer killed susceptible animals, and could be used in his protective inoculations.

Other methods of attenuating the virulence of pathogenic bacteria have since been discovered. Thus, Toussaint has shown that exposure for a short time to a temperature a little below that which destroys the vitality of the pathogenic microörganism, modifies the virulence of a culture so that it may serve for protective inoculations. In the case of Bacillus anthracis, Chauveau has shown that a temperature of 50° C., maintained for eighteen minutes, answers the purpose.

Attenuation of virulence may also be effected by exposure to certain antiseptic agents. This was first ascertained by the writer in experiments made in 1881, the object of which was to determine the comparative value of various disinfecting agents. Incidentally the fact was

brought out that agents which do not completely destroy the vitality of a pathogenic microörganism may cause an attenuation of its pathogenic virulence. In the experiments referred to, the blood of a rabbit recently dead from a form of septicæmia induced by the subcutaneous injection of my own saliva, and due to the presence of a micrococcus (Micrococcus pneumoniæ crouposæ), was subjected to the action of various chemical agents, and subsequently injected into a rabbit to test the destruction of virulence. In the published report of these experiments the following statement is made:

"The most important source of error, however, and one which should be kept in view in future experiments, is the fact that a protective influence has been shown to result from the injection of virus, the virulence of which has been modified, without being entirely destroyed, by the agent used as a disinfectant.

"Sodium hyposulphite and alcohol were the chemical reagents which produced the result noted in these experiments; but it seems probable that a variety of antiseptic substances will be found to be equally effective when used in proper proportion."

Similar results have since been reported by European bacteriologists. Thus, Chamberland and Roux (1883) ascertained that the anthrax bacillus could be attenuated by adding to cultures certain antiseptic agents—carbolic acid, 1 to 800; bichromate of potash, 1 to 100.

Another method of attenuating the virus of a pathogenic microörganism is that recently (1892) discovered by Brieger, Kitasato, and Wassermann. This consists in the cultivation of pathogenic bacteria in a bouillon made from the thymus gland of a calf. It was found that the tetanus bacillus cultivated in this bouillon did not form

spores, and had comparatively little virulence. Mice or rabbits inoculated with it in small doses—0.001 to 0.2 c.c. for a mouse—proved to be subsequently immune. And the blood-serum of an immune rabbit injected into the peritoneal cavity of a mouse (0.1 to 0.5 c.c.) was found to give it immunity from the pathogenic action of a virulent culture of the tetanus bacillus. Similar results were obtained with several other pathogenic bacteria cultivated in the thymus bouillon—spirillum of cholera, bacillus of diphtheria, typhoid bacillus.

Attenuation of virulence may also be effected by cultivating the anthrax bacillus in the body of a non-susceptible animal, like the frog (Lubarsch, Petruschky); or in the blood of the rat (Behring); by exposure to sunlight (Arloing); and by compressed air (Chauveau).

It is a matter of common laboratory experience that many pathogenic bacteria become more or less attenuated when cultivated for a considerable time in artificial media, even when the cultures are renewed at short intervals. This is true of the micrococcus of pneumonia, of streptococcus pyogenes, of the bacillus of diphtheria, of the spirillum of cholera, and, to some extent, of the tubercle bacillus. Indeed, as a general rule, pathogenic bacteria exhibit greater virulence when cultivated in favorable media, and when recently obtained from the body of a susceptible animal; and, on the other hand, pathogenic virulence is diminished by cultivation under unfavorable conditions. Probably similar circumstances produce those differences in the type of epidemic diseases, as to malignancy or comparative mildness, which have been frequently noted; external conditions unfavorable to the development of the specific infectious agent causing an attenuation of virulence, and the reverse. As

pathogenic virulence depends, to a considerable extent at least, upon the formation of toxic substances during the active development of the pathogenic microörganism, we infer that diminished virulence is due to a diminished production of these toxic substances.

An important step was made in the progress of our knowledge in this field of research when it was shown that animals may be made immune against certain infectious diseases by inoculating them with filtered cultures, containing the toxic substances just referred to, but free from the living bacteria to which they owe their origin. The first satisfactory experimental evidence of this important fact was obtained by Salmon and Smith in 1886. These bacteriologists succeeded in producing an immunity in pigeons against the pathogenic effects of the bacillus of hog cholera, which is very fatal to these birds, by inoculating them with sterilized cultures of the bacillus mentioned. Similar results were reported by Roux, in 1888, from the injection into susceptible animals of sterilized cultures of the anthrax bacillus, and also of the bacillus of symptomatic anthrax. More recently (1890) Behring and Kitasato have shown that animals may be made immune against the pathogenic action of the bacillus of tetanus or the bacillus of diphtheria by the injection of filtered, germ-free cultures of these bacilli. Similar results have been obtained by G. and F. Klemperer (1891), in experiments upon rabbits, with filtered cultures of the micrococcus of croupous pneumonia.

In Pasteur's protective inoculations against hydrophobia it is probable that the immunity which is developed after infection by the bite of a rabid animal is due to the toxin (toxalbumin ?) of this disease present in the emulsion of spinal cord which is used in these inoculations.

There is also some evidence to show that a certain degree of immunity against tuberculosis may be produced in guinea-pigs by injections of the toxic substances developed during the growth of the tubercle bacillus—Koch's tuberculin.

Evidently the facts stated have an important bearing upon the *rationale* of acquired immunity, and they appear to support the explanation offered by the writer in a paper published in *The American Journal of the Medical Sciences* in 1881, namely, that immunity depends upon an acquired tolerance to the toxic products of pathogenic bacteria. In the paper referred to I say:

"This explanation is, I believe, to be found in the peculiar properties of the protoplasm, which is the essential framework of every living organism. The properties referred to are: The tolerance which living protoplasm may acquire to certain agents which, in the first instance, have an injurious or even fatal influence upon its vital activity, and the property which it possesses of transmitting its peculiar qualities, inherent or acquired, through numerous generations, to its offshoots or progeny.

"There can be but little doubt that protoplasm is the essential living portion of the cellular elements of animal and vegetable tissues, but as our microscopic analysis of the tissues has not gone beyond the cells of which they are composed, and is not likely to reveal to us the complicated molecular structure of the protoplasm upon which, possibly, the properties under consideration depend, it will be best for the present purpose to limit ourselves to a consideration of the living cells of the body. These cells are the direct descendants of pre-existing cells, and may all be traced back to the sperm-cell and germ-cell of the parents. Now, the view which I am endeavoring to elucidate is, that during a non-fatal attack of one of the specific diseases the cellular elements implicated which do not succumb to the destructive influ-

ence of the poison, acquire a tolerance to this poison which is transmissible to their progeny, and which is the reason of the exemption which the individual enjoys from future attacks of the same disease.

"The known facts in regard to the hereditary transmission, by cells, of acquired properties, make it very easy to believe in the transmission of such a tolerance as we imagine to be acquired during the attack, and if it is shown by analogy that there is nothing improbable in the hypothesis that such a tolerance is acquired, we shall have a rational explanation, not of heredity and the mysterious properties of protoplasm, but of the particular result under consideration.

"The transmission of acquired properties is shown in the budding and grafting of choice fruits and flowers, produced by cultivation, upon the wild stock from which they originated. The acquired properties are transmitted indefinitely, and the same sap which on one twig nourishes a sour crab-apple, on another one of the same branch is elaborated into a delicious pippin.

"Numerous examples in illustration of the same fact may be drawn from the animal kingdom; thus, the same mother may give birth to two children by different fathers; the one may inherit a predisposition to consumption, and the other to insanity; and this inheritance, which only manifests itself at the end of many years, has been transmitted from the original sperm-cells of the respective fathers, through countless generations of cells which have lived and died, leaving their progeny to perform their functions.

"The immunity which an individual enjoys from any particular disease must be looked upon as a power of resistance possessed by the cellular elements of those tissues of his body which would yield to the influence of the poison in the case of an unprotected person. There is every reason to believe that it is upon the living portion of the tissues, or the protoplasm of the body, that the disease-poisons act; for if it were upon non-living

matter—formed material, Beale—and we had to deal only with chemical phenomena, it would be impossible to account for the fact that like causes do not always produce like results. On the other hand, the resistance of living matter to certain destructive influences is a property dependent upon vitality. Thus, living protoplasm resists the action of the bacteria of putrefaction, while dead protoplasm quickly undergoes putrefactive changes. Again, it seems probable that in conditions of debility from age, sickness, starvation, or any other cause, the vital resisting power of the protoplasm is reduced, and certain agents which, under more favorable conditions, would be powerless for harm, may overcome this vital resistance.

"The tolerance to narcotics, opium, tobacco, etc., resulting from a gradual increase of dose, may be cited as an example of acquired tolerance by living protoplasm to poisons, which at the outset would have been fatal in much smaller doses. There can be little doubt that in this instance it is the living protoplasm of the nervous tissues upon which the poison acts to produce its characteristic effects.

"But it is in the specific diseases in which a single attack proves protective that I find the best proof that the cellular elements of the body may acquire a tolerance during the attack which, being transmitted to their cellular progeny, furnishes the protection which the individual enjoys.

"Let us take a particular case. In yellow fever the immediate effect of the poison seems to be to arrest vital processes generally—nutrition, secretion, excretion—and in fatal cases we find that the protoplasm of various organs and tissues has undergone degenerative changes; this is especially true of the liver-cells. Now, we have every reason to believe that this occurs in a less degree in non-fatal cases, but that a sufficient number of cells having resisted the destructive influence of the poison, and become accustomed to its presence, resume their

functions, and that thus the vital processes upon which the life of the individual depends are again carried on in the very presence of the poison, which at first paralyzed or destroyed the vital activity of certain cells. The case is more striking in small-pox, in which there is an undoubted increase of the poison in the tissues during the progress of the disease, but in the first-mentioned disease the patient commonly remains during his sickness in the infected atmosphere, the breathing of which produced the attack from which he is suffering.

"The protection from yellow fever resulting from acclimation—if, indeed, there is such a thing as acclimation independent of an attack of the disease—seems to be a tolerance acquired by repeated exposure to the poison in quantities not sufficient to produce an attack.

"The tolerance enjoyed by the negro race to the malarial poison is probably the result of long residence in malarious regions. Natural selection has doubtless come into play here in establishing this tolerance as a race peculiarity.

"I would, then, place acclimation, inoculation by attenuated viruses, and an attack of any one of the specific diseases, all in the same category, so far as the explanation of the protection afforded is concerned; and, according to my view, the explanation of this phenomenon is to be found in the peculiar properties of living protoplasm which enable it, within certain limits, to adapt itself to varying conditions and injurious influences, and to transmit the impression or modification received in so doing to its offshoots, which continue to perform its functions during the life of the individual."

In my chapter on "Bacteria in Infectious Diseases," in "Bacteria," published in the spring of 1884, I say:

"It may be that the true explanation of the immunity afforded by a mild attack of an infectious germ-disease is to be found in an acquired tolerance to the action of a

chemical poison produced by the microörganism, and consequent ability to bring the resources of nature to bear to restrict invasion by the parasite."

The resources of nature supposed to be brought to bear in restricting invasion by the parasite are referred to on page 15; the explanation offered being the same as that subsequently known as the Metchnikoff theory.

As shown in the discussion of natural immunity, the leucocytes undoubtedly play an important part in the protection of the individual against invasion by pathogenic bacteria. The experiments of Metchnikoff and others show that the introduction of pathogenic bacteria into the subcutaneous tissues of an immune animal often leads to an emigration of leucocytes to the point of invasion far exceeding that which occurs in one which has not a natural or acquired immunity for the particular microörganism thus introduced. This fact, however, does not demonstrate the truth of the theory of phagocytosis, and, as we shall see, recent researches indicate that the principal factor in the production of acquired immunity is the presence in the blood of the immune animal of some substance capable of neutralizing the toxic products of the particular pathogenic microörganism against which immunity exists, or of destroying the "germ" itself.

These substances are called antitoxins. As pointed out by Buchner in a recent paper (*Op. cit.*, p. 24), the antitoxins differ essentially from the so-called alexins, to which natural immunity is ascribed. The alexins are characterized by their germicidal and globulicidal action —they destroy both the red corpuscles and the leucocytes of animals belonging to a different species from that from which they have been obtained, and by their

coagulability and instability—destroyed by sunlight and by a temperature of 50° to 55° C. On the other hand, the antitoxins best known (diphtheria and tetanus) have no germicidal or globulicidal action; they resist the action of sunlight and require a temperature of 70° to 80° C. for their destruction.

Our knowledge of the antoxins dates from the experiments made in the Hygienic Institute of Tokio, by Ogata and Jasuhara, in 1890. These bacteriologists discovered the important fact that the blood of an animal immune against anthrax contains some substance which neutralizes the toxic products of the anthrax bacillus. When cultures were made in the blood of dogs, frogs, or of white rats, which animals have a natural immunity against anthrax, they were found not to kill mice inoculated with them. Further experiments showed that mice inoculated with virulent anthrax cultures did not succumb to anthrax septicæmia if they received at the same time a subcutaneous injection of the blood of an immune animal. Further, it was found that mice which had survived anthrax infection as a result of this treatment were immune at a later date (after several weeks), when inoculated with a virulent culture of the anthrax bacillus. In the same year (1890) Behring and Kitasato discovered that the blood of an animal which has an acquired immunity against tetanus or diphtheria, when added to a virulent culture of one or the other of these bacilli, neutralizes the pathogenic power of such cultures, as shown by inoculation into susceptible animals. And also that cultures from which the bacilli have been removed by filtration, and which kill susceptible animals in very small amounts, have their toxic potency destroyed by adding to them the blood of an immune animal, which is thus

directly proved to contain an antitoxin—which comparative experiments show not to be present in the blood of non-immune animals. In the experiments of Behring and Kitasato referred to, it was found that 5 c.c. of serum from the blood of an immune rabbit, mixed with 1 c.c. of a virulent filtrate of the tetanus bacillus, and allowed to stand for twenty-four hours, completely neutralized its toxic power, as shown by inoculations in mice; 0.2 c.c. of this mixture injected into a mouse was without effect, while 0.0001 c.c. of the filtrate, without such admixture, was infallibly fatal to mice. The mice inoculated with this mixture remained immune for forty or fifty days, after which they gradually lost their immunity. The blood or serum from an immune rabbit, when preserved in a dark, cool place, retained its power of neutralizing the tetanus toxalbumin for about a week, after which time it gradually lost this power. Behring and Kitasato have also shown that the serum of a diphtheria-immune rabbit destroys the potent toxalbumin in diphtheria cultures. It does not, however, possess any germicidal power against the diphtheria bacillus.

Tizzoni and Cattani (1891) have obtained similar results. By repeated inoculations with gradually increasing doses of the tetanus toxin they succeeded in producing immunity in dogs and other animals; and they found by experiment that a small amount of blood-serum from an immune dog completely destroys the toxic power of a filtrate from cultures of the tetanus bacillus—one or two drops of serum neutralized 0.5 c.c. of filtrate after fifteen or twenty minutes' contact. They also ascertained that small amounts of blood-serum from an immune dog injected into white mice produced immunity in these animals. In a subsequent paper, pub-

lished in the same year (1891), the authors named reported that the tetanus antitoxin in blood-serum from an immune dog is destroyed by exposure to a temperature of 68° C. for half an hour, or by contact with acids and alkalies; also that it does not pass through a dialyzing membrane.

Kitasato in the same year (1891) published his important researches upon immunity from tetanus. He produced an immunity in rabbits, which lasted about two months, by inoculating them with the filtrate from a culture of the tetanus bacillus, and subsequently, in the same locality, with 3 c.c. of a one per cent. solution of terchloride of iodine; this last solution was injected subcutaneously in the same dose at intervals of twenty-four hours for five days. Kitasato also ascertained that a small quantity of blood (0.2 c.c.) from an immune rabbit, when injected into the abdominal cavity of a mouse, gave it immunity from the effects of inoculations with the tetanus bacillus. He also made the important discovery that mice which were inoculated with a virulent culture of the tetanus bacillus, and which subsequently, after tetanic symptoms had appeared, received in the cavity of the abdomen an injection of blood-serum from an immune mouse, were preserved from death. The power of the blood of an immune animal to neutralize the tetanus poison was also shown by mixing the filtrate from a virulent culture with blood-serum from an immune animal, and allowing it to stand for twenty-four hours; a dose three hundred times greater than would have sufficed to kill a mouse proved to be without effect after such admixture with blood-serum. Control experiments showed that blood-serum from animals not immune had no effect upon the virulence of the filtrate from

tetanus cultures. The duration of immunity induced in this way was found to be from forty to fifty days.

Vaillard (1891) has succeeded in producing immunity in rabbits by repeated injections into the circulation of filtered cultures which had been exposed for an hour to a temperature of 60° C. At a temperature of 65° C. both the toxic and the immunizing action of the filtrate were destroyed.

In 1891 G. and F. Klemperer published an important memoir, in which they give an account of their researches relating to the question of immunity, etc., in animals subject to the form of septicæmia produced by the micrococcus of croupous pneumonia. They were able to produce immunity in susceptible animals by introducing into their bodies filtered cultures of this micrococcus, and proved by experiment that this immunity had a duration of at least six months. They also arrived at the conclusion that the immunity induced by injecting filtered cultures into susceptible animals is due to the production of an antitoxin in the body of the animal.

Emmerich, at the meeting of the International Congress for Hygiene and Demography, in London (1891), reported results corresponding with those obtained by G. and F. Klemperer as regards the production of immunity. He also gave an account of experiments by Dönissen in which the injection of 20 to 25 c.c. of blood or expressed tissue juices, filtered through porcelain, from an immune rabbit into an unprotected rabbit, subsequently to infection by a bouillon culture of "diplococcus pneumoniæ," prevented the development of fatal septicæmia. Similar results have been reported by Emmerich and Fawitzky in experiments made upon mice with the bacillus of hog erysipelas (rothlauf).

Brieger, Kitasato, and Wassermann have reported (1892) their success in conferring immunity upon guinea-pigs against the pathogenic action of the cholera spirillum. They found that attenuated cultures suitable for use as "vaccines" could be obtained by cultivating the spirillum in bouillon made from the thymus gland of the calf, by which means they have also obtained attenuated cultures of the bacillus of diphtheria, the bacillus of typhoid fever, the bacillus of tetanus, and the streptococcus of erysipelas. Guinea-pigs inoculated with a culture in thymus bouillon, which had been subjected to a temperature of 65° C. for fifteen minutes, were found, after twenty-four hours, to be immune against virulent cultures in twice the amount which would otherwise have been fatal.

During the past two or three years numerous additional experiments have been reported which confirm the results already referred to, and show that immunity may be produced in a similar manner against the toxic products of various other pathogenic bacteria — the typhoid bacillus, the "colon bacillus," streptococcus pyogenes, staphylococcus pyogenes aureus and albus, etc.

The Italian investigators Tizzoni and Centanni, in 1892, published a preliminary communication in which they gave the results of experiments which appear to show that in guinea-pigs treated with tuberculin, by Koch's method, a substance is developed which neutralizes the pathogenic potency of the tubercle bacillus. Professor Tizzoni and his associate, Dr. Schwarz, have also (1892) obtained evidence that there is an antitoxin of rabies. Blood-serum taken from a rabbit having an artificial immunity against this disease was found to neutralize *in vitro* the virulence of the spinal marrow of a rabid

animal after a contact of five hours. The blood-serum of dogs having an acquired immunity against rabies was found to have a similar action, but in much less degree. The substance (antitoxin) present in the blood-serum of an immune rabbit does not dialyze; it is precipitated by alcohol, and preserves its activity, to a considerable extent, after precipitation; it is soluble in glycerin and is said to have the general characters of a "globulin." The experimenters named also succeeded in conferring immunity upon susceptible animals by injecting into them blood-serum containing this antitoxin. According to the Italian investigators named, the antitoxins of tetanus and of rabies are found only in the blood-serum of immune animals and not in the tissues (nervous or muscular), or in the parenchyma of the various organs.

Professor Ehrlich, of Berlin, in 1891, published the results of some researches which have an important bearing upon the explanation of acquired immunity, and which show that susceptible animals may be made immune against the action of certain toxic proteids of vegetable origin, other than those produced by bacteria; also that this immunity depends upon the presence of an antitoxin in the blood-serum of the immune animals.

The experiments of Ehrlich were made with two very potent toxalbumins, one—ricin—from the castor-oil bean, the other—abrin—from the jequirity bean. The toxic potency of ricin is somewhat greater than that of abrin, and it is estimated by Ehrlich that 1 gm. of this substance would suffice to kill one and a half million of guinea-pigs. When injected beneath the skin in dilute solution it produces intense local inflammation, resulting in necrosis. Mice are less susceptible than guinea-pigs, and are more easily made immune. This is most readily

accomplished by giving them small and gradually increasing doses with their food. As a result of this treatment the animal resists subcutaneous injections of 200 to 400 times the fatal dose for animals not having this artificial immunity. The fatal dose of abrin is about double that of ricin. When injected into mice in the proportion of 1 c.c. to 20 gm. of body-weight, a solution of 1 part in 100,000 of water proved to be a fatal dose. The local effects are also less pronounced when solutions of abrin are used. These consist principally of an extensive induration of the tissues around the point of injection, and a subsequent falling off of the hair over the indurated area. When introduced into the conjunctival sac, however, abrin produces a local inflammation in smaller amounts than ricin, a solution of 1 to 800 being sufficient to cause a decided, but temporary, conjunctivitis. Solutions of 1 to 50, or 1 to 100, of either of these toxalbumins, introduced into the eye of a mouse, gave rise to a panophthalmitis which commonly resulted in destruction of the eye. But in mice which have been rendered immune, by feeding them for several weeks with food containing one of these toxalbumins, no reaction follows the introduction into the eye of the strongest possible solution, or of a paste made by adding abrin to a ten per cent. salt solution. Ehrlich gives the following explanation of the remarkable degree of immunity established in his experiments by the method mentioned:

"All of these phenomena depend, as may easily be shown, upon the fact that the blood contains a body—antiabrin—which completely neutralizes the action of the abrin, probably by destroying this body."

In a later paper (1892) Ehrlich has given an account of subsequent experiments which show that the young of

mice which have an acquired immunity for these vegetable toxalbumins may acquire immunity from the ingestion of their mother's milk; and also, that immunity from tetanus may be acquired in a brief time by young mice through their mother's milk. In his tetanus experiments Ehrlich used blood-serum from an immune horse to give immunity to the mother-mouse, when her young were already seventeen days old. Of this blood-serum 2 c.c. was injected at a time on two successive days. The day after the injection one of the sucklings received a tetanus inoculation, by means of a splinter of wood to which spores were attached. The animal remained in good health, while a much larger control mouse, inoculated in the same way, died of tetanus at the end of twenty-six hours. Other sucklings, inoculated at the end of forty-eight hours and of seventy-two hours after the mother had received the injection of blood-serum, likewise remained in good health, while the control mice died.

The possibility of conferring immunity by means of the milk of an immune animal is further shown by the experiments of Brieger and Ehrlich (1892). A female goat was immunized against tetanus by the daily injection of "thymus-tetanus bouillon." The dose was gradually increased from 0.2 c.c. to 10 c.c. At the end of thirty-seven days a mouse, which received 0.1 c.c. of the milk of this goat in the cavity of the abdomen, proved to be immune against tetanus. Further experiments gave a similar result, even when the milk of the goat was not injected into the peritoneal cavity of the mouse until several hours after inoculation with a virulent culture of the tetanus bacillus.

When the casein of the milk was separated it retained

its full immunizing activity, and by concentration *in vacuo* a thick milk was obtained which had a very high immunization value—0.2 c.c. of this milk protected a mouse against forty-eight times the lethal dose of a tetanus culture.

In a subsequent communication (1893) Brieger and Ehrlich describe their method of obtaining the antitoxin of tetanus from milk in a more concentrated form. They found by experiment that it was precipitated by ammonium sulphate and magnesium sulphate. From twenty seven to thirty per cent. of ammonium sulphate added to milk caused a precipitation of the greater part of the antitoxin. This precipitate was dissolved in water, dialyzed in running water, then filtered and evaporated in shallow dishes at 35° C. in a vacuum. One litre of milk from an immune goat gave about 1 gm. of a transparent, yellowish-white precipitate, which contained fourteen per cent. of ammonium sulphate. This precipitate had from four hundred to six hundred times the potency of the milk from which it was obtained in neutralizing the tetanus toxine.

In a still later communication (1893) Brieger and Cohn give an improved method of separating the antitoxin from the precipitate thrown down with ammonium sulphate. The finely pulverized precipitate is shaken up with pure chloroform, and when this is allowed to stand the antitoxin rises to the surface while the ammonium salt sinks to the bottom. By filling the vessel to the margin with chloroform, the antitoxin floating on the surface can be skimmed off, after which it quickly dries. By this method the considerable loss which occurred in the dialyzer, used in the previously described method, is avoided.

A most interesting question presents itself in connection with the discovery of the antitoxins. Does the animal which is immune from the toxic action of any particular toxalbumin also have an immunity for other toxic proteids of the same class? The experimental evidence on record indicates that it does not. In Ehrlich's experiments with ricin and abrin he ascertained that an animal which had been made immune against one of these substances was quite as susceptible to the toxic action of the other as if it did not possess this immunity, *i.e.*, the antitoxin of ricin does not destroy abrin, and *vice versa*. As an illustration of the fact, he states that in one experiment a rabbit was made immune for ricin to such an extent that the introduction into its eye of this substance in powder produced no inflammatory reaction; but the subsequent introduction of a solution of abrin, of 1 to 10,000, caused a violent inflammation. In this connection we may remark that there is some evidence to show that persons who are repeatedly stung by certain poisonous insects—mosquitoes, bees—acquire a greater or less degree of immunity from the distressing local effects of their stings.

We have also experimental evidence that animals may acquire a certain degree of immunity from the toxic action of the venom of the rattlesnake. This was first demonstrated by Sewall (1887), and has been recently confirmed by Calmette (1894). In his paper detailing the results of his experiments the author last named says:

"Animals may be immunized against the venom of serpents either by means of repeated injections of doses at first feeble and progressively stronger, or by means of successive injections of venom mixed with certain chemi-

cal substances, among which I mention especially chloride of gold and the hypochlorites of lime or of soda."

"The serum of animals thus treated is at the same time preventive, antitoxic, and therapeutic, exactly as is that of animals immunized against diphtheria or tetanus."

"If we inoculate a certain number of rabbits, under the skin of the thigh, with the same dose, 1 milligr. of cobra venom for example, and if we treat all of these animals, with the exception of some for control, by subcutaneous or intraperitoneal injections of the serum of rabbits immunized against 4 milligrs. of the same venom, all of the control animals not treated will die within three or four hours, while all of the animals will recover which receive 5 c.c. of the therapeutic serum within an hour after receiving the venom."

The experimental evidence recorded justifies the conclusion that, in the diseases referred to, acquired immunity depends, chiefly at least, upon the presence of a peculiar proteid substance in the blood of the immune animal—antitoxin—which neutralizes the toxic substance —toxin or toxalbumin—to which the morbid phenomena which characterize the disease are due.

But it would be premature to infer that in all infectious diseases immunity depends upon the production of an antitoxin in the blood of the immune animal. Indeed we have experimental evidence which shows that in certain cases the blood-serum of immune animals has no antitoxic power, but acts upon the germ itself, instead of upon its toxic products.

As a rule the antitoxins have no bactericidal action; but it has been shown by the experiments of Gamaleïa, Pfeiffer, and others, that in animals which have an acquired immunity against the spirillum of Asiatic cholera and against spirillum Metchnikovi, there is a decided in-

crease in the bactericidal power of the blood-serum, and that immunity probably depends upon this fact.

The researches of Metchnikoff upon hog cholera, of Issaeff upon pneumonia, and of Sanarelli upon typhoid fever indicate that the immunity conferred upon susceptible animals by protective inoculations is not due to an antitoxin but to a substance present in the blood of immune individuals which acts directly upon the pathogenic microörganism, as is the case in cholera-immune animals. The animals immunized are said to be quite as sensitive to the action of the bacterial poisons as are those which have not received protective inoculations. "Their serum does not protect against the toxin, but against the microbe" (Roux).

Certain important questions present themselves in connection with the production of antitoxins and germicidal substances in the blood of immune animals, one of which is: Is the production of the antitoxin continuous while immunity lasts, or does it occur only during the modified attack which results from inoculation with an attenuated virus, or of filtered cultures, the antitoxin being subsequently retained in the circulating blood? The latter supposition does not appear very plausible, but it must be remembered that these antitoxins do not dialyze — *i.e.*, they do not pass through animal membranes—and consequently would not readily escape from the blood-vessels, notwithstanding the fact that they are held in solution in the circulating fluid. On the other hand, the passage of the tetanus antitoxin into the mother's milk would indicate a continuous supply, otherwise the immunity of the mother would soon be lost. Further experiments are required to settle this question in a definite manner, and also to determine the exact

source of the antitoxins in the animal body and the *modus operandi* of their production. According to Buchner (1894) the antitoxins are not to be regarded as reactive products developed in the body of the immune animal, but as modified, changed, and "*entgiftete*" products of the specific bacterial cells. He insists that they do not neutralize the toxins by direct contact, but only through the medium of the living organism. This explanation scarcely appears tenable in view of the experimental evidence, and the fact that the antitoxin of tetanus escapes in considerable quantity with the milk of an immune goat without, apparently, diminishing the immunity of the animal. In the immunity against the toxic action of the vegetable toxalbumins—ricin and abrin—as shown by Ehrlich's experiments, there are no "products of bacterial cells" introduced with the pure toxalbumin from the castor bean or the jequirity bean; and we have sufficiently numerous experiments to show that immunity, with the presence of antitoxins in the blood, may be induced by precipitated and purified toxalbumins from filtered cultures. Several of the experimenters, also, have reported that the toxins from bacterial cultures are neutralized *in vitro* by blood-serum from an immune animal, or by the precipitated antitoxin from such serum after contact for a certain number of hours. If they are correct in the statement that a certain time is required after the antitoxin has been brought in contact with the toxin, in order that the latter may be neutralized, as shown by injection of the mixture into a susceptible animal, then we must admit that this neutralizing effect occurs outside of the body of the animal, as has been generally assumed.

The experiments of Vaillard are also opposed to

Buchner's view. He reports that in a rabbit immunized against tetanus, "a volume of blood equal to the total amount which circulates in its body may be withdrawn without diminishing, in an appreciable manner, the antitoxic power of its serum. Therefore the antitoxin must be reproduced as fast as it is withdrawn." The author from whom we have just quoted (Roux) also reports the results of experiments which show that the antitoxic value of the serum of a rabbit immunized against tetanus does not bear a direct relation to the quantity of the tetanus toxin introduced, but depends also upon the method adopted. When a few large doses are given the result is far less favorable than that obtained by giving the same amount in repeated small doses. The serum of an animal immunized by thirty-three small doses was found to neutralize, *in vitro*, 150 parts of toxin, while that of an animal which received the same amount in nine doses, only neutralized 25 parts of the same toxin. On the other hand we have experiments which indicate that the supposed neutralization of a toxin by an antitoxin, *in vitro*, is not really a chemical neutralization. Thus Buchner found in his experiments with the tetanus toxin and antitoxin, in a dry powder, that when mixed in a certain proportion and injected into white mice no tetanic symptoms were induced. But the same mixture gave rise to distinct tetanic symptoms in guinea-pigs, showing that the inference that the toxin had been neutralized *in vitro*, based upon the experiment on mice, would have been a mistake. And certain observations made by Roux and Vaillard seem to give support to the view that neutralization does not occur *in vitro*, but that the result depends upon some physiological reaction induced by the antitoxin within the body of the living animal. These

bacteriologists found that when the antitoxin was apparently in excess, tetanic symptoms could be induced in susceptible animals if they had been in any way exhausted prior to the injection of the mixture of toxin and antitoxin; and that the same result followed when their resisting power had been reduced by injecting into them at the same time filtered cultures of other bacteria.

In this connection the results reported by Calmette, Phisalix, and Bertrand are of interest. These investigators found that when the antitoxin of snake-poison was mixed with this venom in a proportion which neutralized its toxic properties, as shown by experimental inoculations, and the mixture then heated to 70° C., by which temperature the antitoxin is destroyed, subsequent inoculations showed that the toxin was still active.

The experiments of Stern (1894) show that the typhoid bacillus not only grows in blood-serum from a typhoid convalescent, which has been proved to neutralize its pathogenic effects when injected into a susceptible animal, but also that its toxic products are developed in this culture medium. From this Stern concludes that the serum must in some way act upon the infected animal, causing changes which enable it to resist infection, rather than upon the bacillus or upon its toxic products directly. It has also been shown by Behring (1890) for the diphtheria bacillus, by Vaillard for the tetanus bacillus (1892), and by Issaeff (1893) for the micrococcus of pneumonia, that these several pathogenic microörganisms may be cultivated in the blood-serum of animals immunized for the diseases which they produce.

We must admit that the exact source and method of production of the antitoxins in the animal body, and

their mode of action, are still undetermined; and, for the present, we must be satisfied with the knowledge that in some way these so-called antitoxins, which have been proved to be present in the blood-serum of immune animals protect these animals from infection by pathogenic bacteria. And that when transferred to susceptible animals they confer upon them a temporary immunity; or if introduced after infection may neutralize the pathogenic action of the toxins produced by specific "disease germs."

Finally, there is experimental evidence to show that immunity from the pathogenic action of certain bacteria may be produced by previous injections of cultures of other bacteria (sterilized or otherwise), and even by the injection of the blood-serum of normal individuals or of other substances.

Pasteur, in 1880, communicated to the French Academy of Sciences the results of experiments which led him to the conclusion that fowls which had an acquired immunity against chicken cholera also had an immunity against anthrax. And Roux has recently reported that the blood-serum of a horse which has been immunized against tetanus neutralizes the toxic power of cobra poison. But the contrary effect is not produced—*i.e.*, the blood-serum of an animal immunized against the cobra poison does not neutralize the tetanus toxalbumin. The statement is also made that the blood-serum of a rabbit which has been made immune against hydrophobia will protect a susceptible animal against the cobra venom in doses four or five times as large as the usually lethal dose. Also that rabbits which have been immunized against snake-poison are less susceptible to the toxic effects of abrin and the reverse—*i.e.*, antiabrin

neutralizes, to some extent at least, the toxic action of snake-poison.

The writer in his "Report on the Etiology and Prevention of Yellow Fever" (1890), gives, on pp. 196 and 197, experimental evidence which shows that the injection into the peritoneal cavity of rabbits of cultures of Bacillus pyocyaneus or of Bacillus gracilis, protected the animals from the fatal results of subsequent injections of my bacillus X, which was extremely fatal to rabbits when injected into the cavity of the abdomen in doses of 1 or 2 c.c. In referring to these experiments I say: "The evidence favors the view that death results from peritonitis (and toxæmia?) induced by intra-peritoneal injections, and that *a tolerance on the part of the peritoneum may be established by the injection of certain other bacilli, or of sterilized cultures of bacillus X.*"

This corresponds with facts subsequently developed by Issaeff (1894) in his experiments with reference to immunity in guinea-pigs against cholera cultures injected into the cavity of the abdomen. He found that a certain degree of immunity was established by the previous injection of blood-serum from normal individuals, and also of various acids, alkalies, and neutral liquids. The immunity produced in this way was, however, feeble and temporary, and could not properly be considered as identical with that produced by inoculations with attenuated cultures which give rise to a mild attack of a specific disease.

Referring to my observation that injections of a culture of Bacillus pyocyaneus, either sterilized or otherwise, gave rabbits an immunity from the pathogenic action of my bacillus X, I may call attention to the more recent experiments of Rumpf (1893) and of Kraus and Buswell

(1894), with reference to the treatment of typhoid fever with sterilized cultures of this bacillus. And in this connection would remark that my bacillus X belongs to the same group as the typhoid bacillus and Bacillus coli communis, but is more decidedly pathogenic than either of these. Cesaris-Demel and Orlandi have recently (1894) reported their success in immunizing animals against infection by the typhoid bacillus by means of sterilized cultures of Bacillus coli communis, and the reverse.

While this chapter relates especially to acquired immunity from infectious diseases, and this immunity has been shown to depend, in a number of these diseases at least, upon the development of antitoxins in the body of the immune animal, it may be worth while to refer briefly, before closing, to some examples of acquired immunity of a different order. We refer to the tolerance of extremes of heat and cold which may be established by habitual exposure, and, more especially, to the tolerance to narcotics and irritant poisons, which is very remarkable and has never been explained in a satisfactory manner. A recent writer (Samuel, 1892) has presented experimental evidence which shows that the local inflammation which results from the application of croton-oil to the ear of a rabbit does not occur when a second application is made to the same ear after recovery from the effects of the first. That a tolerance may be acquired to comparatively large doses of arsenic is well known, and the tolerance which the victims of drug habits acquire to enormous doses of narcotics is a matter of daily observation. In the writer's paper on acquired immunity, published in 1881, from which extended quotations have been made (pp. 41–45), an attempt is made to account for acquired immunity in infectious diseases as

analogous to the immunity to drugs just referred to; but the experimental evidence presented in the present chapter shows that the analogy has no scientific foundation in the absence of any evidence that there is an antitoxin of morphia, of cocaine, of narcotin, etc., in the blood of the *habitués* of these drugs.

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PART SECOND.

PROTECTIVE INOCULATIONS IN INFECTIOUS DISEASES AND SERUM-THERAPY.

I.

ANTHRAX.

The discovery of the anthrax bacillus by Davaine (1863), and the demonstration of its etiological relation to the disease with which it is associated by the researches of Pasteur, Toussaint, Koch (1878–1881), and other pioneers in this field of investigation, constitute the foundation of our present knowledge of bacteriology and of the practical results attained in protective inoculations and serum-therapy. And a review of the literature relating to the anthrax bacillus would show, in a most interesting manner, the successive steps by which we have arrived at the important results which have gone so far toward establishing medicine upon a scientific basis. In the present volume, however, we must confine our attention to those investigations which relate directly to the subject in hand.

Toussaint, a pioneer in researches relating to protective inoculations, has a short paper in the *Comptes-Rendus of the French Academy of Sciences* of July 12, 1880, entitled "Immunity from Anthrax ("charbon") Acquired as a Result of Protective Inoculations."

In this paper he announces his discovery of the important fact that the anthrax bacillus does not form spores in the tissues or liquids of the body of an infected animal, but multiplies alone by binary division—"*sa multiplication se fait toujours par une division du mycélium.*"

In the same communication he reports his success in conferring immunity upon five sheep by means of protective inoculations, and also upon four young dogs. We must therefore accord him the priority in the publication of experimental data demonstrating the practicability of accomplishing this result.

Toussaint does not give his method in the communication above referred to, but the following quotation from a communication made to the Academy of Sciences on March 19, 1881, by Pasteur, shows the method, and at the same time demonstrates the fact that Toussaint was the first to produce immunity by the use of sterilized cultures. Pasteur says:

"By inoculating sheep either with defibrinated blood from an animal dead of anthrax, after filtration through several thicknesses of paper, or with the same blood defibrinated and subjected to 55° C. for ten minutes, according to Toussaint, these sheep subsequently resist inoculations with anthrax blood. . . . The bacillus, according to Toussaint, deposits in the blood of animals in which it multiplies a substance which may become its own vaccine. By filtration while cold in one case, by a temperature of 55° C. in the other, the bacillus is said to be removed or killed; so that the inoculation of filtered or heated blood introduces into the animal inoculated vaccinal matter deprived of bacteria."

After thus stating Toussaint's method and explanation Pasteur proceeds to raise objections against this method, the principal of which is that the anthrax bacillus is not killed by exposure to a temperature of 55° C. for ten minutes, and that inoculation with a virus prepared in this way would result in a considerable mortality among the animals inoculated, although those surviving the inoculation would be protected.

In a communication made to the French Academy of Sciences, September 27, 1880, Pasteur gave an account of an experiment made July 14, 1879, upon two cows, which in connection with a subsequent experiment, made August 6, 1880, upon four cows, led him to the conclusion that a single attack of anthrax protects from subsequent attacks. He says in the paper referred to:

"On the 15th of September, 1880, two cows, A and C, which had been very ill as a result of the first inoculation, made August 6th, were reinoculated on the left side, that is to say, on the side opposite the first inoculation. We used five drops of culture of the bacillus of anthrax ('*bactéridies du charbon*'). The following days there was no perceptible œdema and no elevation of temperature in either cow. The question is then resolved: a single attack protects ('*le charbon ne récidive pas*')."

The next important steps in the line of experimental research leading to protective inoculations in the disease under consideration were reported by Pasteur in his communication to the French Academy made at the séance of February 28, 1881 (with the collaboration of Chamberland and Roux), entitled "De l'atténuation des virus et de leur retour à la virulence." In this connection Pasteur announces his discovery of the fact that when cultivated at a temperature of 42° to 43° C., the anthrax bacillus no longer forms spores and rapidly loses its virulence. He says:

"As regards its virulence, the extraordinary fact has been ascertained that the bacillus is no longer virulent after it has been kept for eight days at a temperature of 42° to 43° C.; at least its cultures are inoffensive for the guinea-pig, the rabbit, and the sheep, three species of animals which are very susceptible to anthrax. We are

able, then, not only to attenuate virulence but to effect its complete extinction, by a simple method of cultivation.

"Before the extinction of its virulence the microbe of charbon passes through the intermediate degrees of attenuation, and, on the other hand, as happens also with the microbe of fowl cholera, each of these degrees of virulence may be reproduced by cultivation. Finally, as shown in one of our recent communications, since one attack of anthrax protects, each one of our attenuated microbes of charbon constitutes a vaccine for the microbe of superior virulence; that is to say, a virus suitable to produce a more benign malady. What, then, is more easy than to find among these a virus suitable to give anthrax to sheep, cows, or horses, without causing them to perish, and capable of preserving them from a subsequent fatal attack? We have already practised this operation upon sheep with great success."

At the end of this important communication Pasteur says:

"I concluded my communication of October 26th by remarking that the attenuation of virus by the influence of the air is probably one of the factors in the extinction of great epidemics. The facts just recorded, in their turn, may serve to explain the so-called spontaneous appearance of these scourges. An epidemic which has been terminated by the attenuation of its virus may be relighted by the reinforcement of this virus under certain influences. The accounts which I have read of the spontaneous appearance of the plague appear to me to offer examples of this. The plague is a virulent malady which prevails in certain countries. In all of these countries its attenuated virus probably exists, ready to take its active form when the necessary conditions as to climate, famine, and distress again prevail. There are other virulent maladies which appear spontaneously in all countries, such as camp typhus. Without doubt the germs of the microbes which cause these diseases are everywhere distrib-

uted. Man carries them about him, or in his intestine, without great damage, but ready, nevertheless, to become dangerous when, as a result of certain conditions or of successive development upon the surface of wounds, in bodies enfeebled or otherwise, their virulence is progressively reinforced. And from this point of view virulence appears to us under a new light which is somewhat disquieting for humanity, unless nature, in the evolution which has occurred during the past centuries, has already encountered all possible occasions for the production of virulent or contagious diseases, an assumption which seems very improbable.

"What is an inoffensive microscopic organism for man or for a given animal? It is an organism which cannot develop in our body or in that of the animal; but nothing proves that if this microscopic organism should penetrate into some other of the thousands of species of the creation it could not invade it and cause it to become sick. Its virulence, then, reinforced by passing through a series of individuals of this species, might become such that it could invade man or one of the domestic animals. By this means new contagions may be created. I am disposed to believe that it is in this way that, in the course of ages, have appeared small-pox, syphilis, the plague, yellow fever, etc."

This broad induction has received considerable support from more recent researches, which show that the typhoid bacillus, the cholera spirillum, and other important pathogenic bacteria become attenuated when they lead a saprophytic existence for some time, and regain their virulence when they are propagated within the bodies of susceptible animals.

In a later communication (March 21, 1881) Pasteur says that he has found by experiment that when attenuated varieties of the anthrax bacillus form spores, these again reproduce the same pathogenic variety, so that

cultures of each degree of attenuation can be maintained indefinitely.

On June 13, 1881, Pasteur communicated the results of his famous experiment at Pouilly-le-Fort, near Melun. He says:

"On the 5th of May, 1881, we inoculated, by means of a Pravaz syringe, twenty-four sheep, one goat, and six cows, each animal with five drops of an attenuated culture of the anthrax bacillus. On the 17th of May we re-inoculated these animals with a second virus, also attenuated, but more virulent than the first.

"On the 31st of May we proceeded to make a very virulent inoculation in order to test the efficacy of the preventive inoculations made on the 5th and 7th of May. For this experiment we inoculated the thirty vaccinated animals, and also twenty-four sheep, one goat, and four cows, which had not received any previous treatment.

"The very virulent virus used on the 31st of May was obtained from spores preserved in my laboratory since the 21st of March, 1877.

"In order to make the experiments more comparable we inoculated alternately a vaccinated and a non-vaccinated animal. When the operation was finished all of those present were invited to reassemble on June 2d, *i.e.*, forty-eight hours after the virulent inoculation was made.

"Upon the arrival of the visitors on June 2d, all were astonished at the result. The twenty-four sheep, the goat, and the six cows which had received the attenuated virus, all presented the appearance of health. On the contrary, twenty of the sheep, and the goat, which had not been vaccinated, were already dead of anthrax; two more of the non-vaccinated sheep died before the eyes of the spectators, and the last of the series expired before the end of the day. The non-vaccinated cows were not dead. We had previously proved that cows are less subject than sheep to die of anthrax. But all had an extensive œdema at the point of inoculation, behind the

shoulder. Certain of these œdematous swellings increased during the following days to such dimensions that they contained several litres of liquid, deforming the animal. One of them even nearly touched the earth. The temperature of these cows was elevated 3° C. The vaccinated cows did not experience any elevation of temperature, or tumefaction, or the slightest loss of appetite. The success, therefore, was as complete for the cows as for the sheep."

The fact that infection depends to some extent upon the number of bacilli introduced, and that animals which have a certain degree of immunity, like the Algerian race of sheep, may succumb when they are inoculated with a certain quantity of virus, although they resist a smaller amount, was announced by Chauveau in his communication to the French Academy at the séance of June 28, 1880. He says:

"The facts which I have just presented show that the anthrax bacillus behaves in the organism of Algerian sheep not as if it were deprived of the principles necessary for its development, but rather as if it were in a medium rendered unsuitable for its growth by the presence of substances injurious to it. In a very small number the bacilli are arrested in their development by the inhibitory influence of these substances. When they are very numerous, on the contrary, they surmount more easily this obstacle to their proliferation."

This quotation shows that Chauveau had at this early date arrived at an explanation of immunity very nearly in accord with that which is now generally accepted.

The fact that infection is influenced by the quantity of the infectious material introduced had previously been insisted upon by Davaine in his paper entitled "*Recherches sur quelques unes des conditions qui favorisent ou qui em-*

pêchent le dévelopement de la septicémie," published in the *Bulletin of the Academy of Medicine*, séance of February 18, 1879.

Davaine says:

"A third condition relates to the quantity of bacteria introduced into the tissues. This question of quantity has been made manifest in our experiments. Not only does it differ in different species of animals, the rabbit and the dog, for example, but it varies in different animals of the same species."

In his communication to the Academy of Sciences, made on April 4, 1881, Chauveau gives the results of his experiments in producing immunity by inoculations with very small quantities of virus. After some preliminary experiments with a larger number, five sheep were inoculated with diluted anthrax blood estimated to contain two hundred and fifty bacilli for each. All of the animals survived the inoculation after having manifested some slight febrile reaction. Six weeks later all were reinoculated with a dose which should have been fatal to an unprotected animal. One of the animals died of anthrax, the other four resisted perfectly.

On June 26, 1882, Chauveau reported to the Academy of Sciences the results of his experiments relating to the protection of animals from anthrax infection by the method of Toussaint. By carefully conducted experiments Chauveau found that nine or ten minutes' exposure to a temperature of 54° C. killed all of the bacilli in anthrax blood, and the same result was obtained by sixteen minutes' exposure to 52° C., while at 50° C. the time required is twenty minutes. An attenuated virus suitable for protective inoculations is obtained by exposure for a somewhat shorter time, and as a result of his experi-

ments Chauveau was led to the conclusion that for a first inoculation anthrax blood heated to 50° C. for fifteen minutes afforded a good attenuated virus. This was to be followed after an interval of ten to fifteen days by a second inoculation with a stronger virus, obtained by exposing anthrax blood to the same temperature (50° C.) for nine or ten minutes. These inoculations sufficed to protect the animals when they were subsequently inoculated with virus of full strength—blood from an animal which had recently succumbed to the disease. Chauveau says with reference to this method:

"In one hour, with a single guinea-pig [dead of anthrax], it is easy to prepare the quantity of vaccine required to inoculate more than five hundred sheep. The inoculation is made with the point of a lancet, charged, by the method in use in my laboratory, with a very small quantity of virus. Two or three large punctures under the skin, upon the internal surface of the ear, suffice for a successful inoculation.

"The vaccine prepared in this way should be used at once, or at least not later than the day after it has been prepared. Experience has shown me that it is then quite as harmless and quite as efficacious as Pasteur's vaccine."

In the preparation of an attenuated virus by this method Chauveau insists upon attention to the following points:

"The first rule to follow, and the principal one, is to practise the heating in such a manner that all parts of the anthrax blood are raised to the required temperature and withdrawn from it at the same instant. When the quantity of blood to be transformed to a vaccine is too great, all parts are not uniformly acted upon by the very short exposure to heat; the virulent agents in the deeper layers may, in that case, preserve all of their activity,

and cause a fatal infection. To avoid this it is best to enclose the blood in little cylindrical pipettes, 1 mm. in diameter. The extremity of these pipettes is sealed, and the portion which contains the blood is immersed in a considerable quantity of water maintained at the proper temperature. At the end of the proper time they are taken from the hot bath and plunged into cold water.

"Another rule should be rigorously observed if one wishes to be sure of success. The blood should be collected under conditions which make it sure that the virulent agents introduced into the tubes all have the same vitality, the same activity, and that they are impressed in the same degree by the heating. This is the case when we take the blood from a guinea-pig just dead, after having survived from thirty-six to forty-eight hours an inoculation with very active virus. Before introducing the blood into the pipettes it should be allowed to coagulate, and the coagula should be broken and crushed in order to obtain a defibrinated blood, which is always very rich in virulent bacilli."

In a subsequent communication (February 26, 1883), Chauveau admits that the application of this method is somewhat difficult and delicate when blood is employed, and states that it is far more satisfactory to use pure cultures, which may be attenuated in the same way. He prefers to cultivate the bacillus in a bouillon made from the flesh of a chicken, and to start his culture by adding to this bouillon a drop of blood from an animal just dead from anthrax. The culture is left for twenty hours in an incubating oven at a temperature of 43° C. During this time there is an abundant development of the bacillus, and the culture is ready to be subjected to the attenuating action of a higher temperature. This is accomplished by exposure to a temperature of 47° C. for a period of one, two, three, or four hours, according to the degree of

attenuation desired. After three hours' exposure the attenuated culture no longer kills guinea-pigs. In a later communication (March 5, 1883) Chauveau states that he has ascertained by experiment that the degree of attenuation produced by this method is maintained in subsequent cultures made at 43° C., from the attenuated culture thus obtained.

Another method of attenuating the virulence of anthrax cultures is that described by Chauveau, in 1885. This consists in cultivating the bacillus at a temperature of 38° to 39° C., under a pressure of eight atmospheres. Cultures treated in this way killed guinea-pigs, but did not kill sheep, cattle, or horses, and constituted a suitable attenuated virus for protective inoculations in these animals. One drop was used for a sheep, and two drops for a cow or a horse, and the immunity was proved to last for a year.

Kitt, in experiments made in 1884 and 1885, found that an attenuation of the virulence of anthrax bacilli may be effected by passing them through birds, which have but little susceptibility to anthrax infection ; but the results obtained were not uniform, and the method was not thought to have any great practical value. In the same paper Kitt gives an account of his experiments with Pasteur's vaccine, No. 1 and No. 2, which he obtained from the agent in Paris. These experiments led him to the conclusion that the attenuated cultures used by Pasteur are too weak. But by passing them through guinea-pigs their virulence was increased so that they served to protect cattle and sheep, although not without danger for the last-mentioned animals.

During the year 1882 Pasteur's method was extensively practised in the department of Eure-et-Loir, where

anthrax was very prevalent and had been the cause of extensive losses. The results of these protective inoculations were reported to the Academy of Sciences (séance of December 18, 1882) by Pasteur, who submitted, with some remarks, a report prepared by M. Boutet, from which we quote as follows:

"The number of sheep vaccinated during the year has been 79,392; among these flocks the average annual loss during the past ten years was 7,237—9.01 per cent. Since the vaccinations but 518 animals have died—0.65 per cent. We must observe that this year, probably on account of the great humidity, the mortality in Eure-et-Loir has only been three per cent. The losses should therefore have been 2,382, instead of 518, without the vaccinations. In the flocks which were only partly vaccinated we had 2,308 vaccinated, and 1,659 not vaccinated; the loss among the first was 8, or 0.4 per cent.; among the second the loss was 60, or 3.9 per cent. We call attention to the fact that in these flocks, in different cantons of the department, the sheep vaccinated and not vaccinated were subjected to the same conditions of soil, of lodging, of food, of temperature, and that consequently they were exposed to identical influences.

"The veterinary surgeons in Eure-et-Loir have vaccinated 4,562 animals of the bovine species. Out of this number the annual loss had been 322. Since the vaccinations only 11 cows have died. That is, the annual mortality has been reduced from 7.03 per cent. to 0.24 per cent.

"Some engorgements, generally not serious, having occurred after vaccinating horses, and the mortality not being great in this species, the veterinarians have thought it prudent not to vaccinate horses, on a large scale. Only 524 were vaccinated; three of these died after the first vaccination."

Notwithstanding this favorable report some bacteriologists, and notably Koch, were not disposed to admit

the practical value of Pasteur's anthrax inoculations. At the conclusion of an elaborate memoir published in the second volume of "Mittheilungen" of the Imperial Board of Health of Germany (1884), Koch and his collaborators (Gaffky and Loeffler) say:

"As now a certain immunity against inoculated anthrax cannot be obtained by the method of Pasteur, as we have seen, without considerable losses, and as the immunity secured at the expense of considerable loss is only an imperfect protection against contracting anthrax in the ordinary way, we must consider the protective inoculations heretofore practised as of doubtful utility, especially when we remember that the second inoculation with a yet stronger virus causes the death of more animals which may serve to further spread the disease."

The attenuating influence of light on the anthrax bacillus and the fact that cultures attenuated in this way may be used for protective inoculations was first ascertained by Arloing (1886). Roux subsequently (1887) showed that the presence of oxygen is a necessary factor in the sterilization of cultures by exposure to sunlight. Behring, who has since been so active in the field of research to which the present volume relates, published an article in the *Centralblatt für klinische Medicin* in 1888 (September 22d) in which he attempted to explain the natural immunity of white rats against anthrax infection. His conclusions are given as follows:

"1. The blood-serum of white rats is not a favorable medium for the anthrax bacillus."

"2. The blood-serum of rats differs from that of animals susceptible to infection by its greater alkalinity."

"3. By the addition of an acid to the blood-serum of rats this becomes a favorable medium for the growth of the anthrax bacillus."

"4. The blood-serum of rats which are treated, during life, in such a way as to reduce the alkalinity of the blood becomes a suitable medium for the development of the anthrax bacillus."

As we have pointed out in the chapter on Natural Immunity (p. 28), the true explanation of the facts ascertained in Behring's experiments is probably to be found, not in the germicidal power of the comparatively small amount of alkali present in the rat's serum, but in the fact that the germicidal proteid produced by the leucocytes is only soluble in an alkaline medium. In a paper published in the *Annales of the Pasteur Institute* (August, 1888), Roux and Chamberland have given an account of experiments made by them which establish the fact that immunity against anthrax may be established by inoculating susceptible animals with blood from an animal dead from anthrax, in which the anthrax bacilli had been killed by heat or removed by filtration (Sur l'immunité contre le charbon conférée par des substances chimiques). These experiments were commenced in 1881. The authors named say:

"In repeating the experiments of Toussaint upon anthrax blood which had been heated, we made several observations which convinced us that it is possible to confer immunity against anthrax upon sheep by injecting under their skin anthrax blood which does not contain any living bacilli."

While immunity was produced in this way, Roux and Chamberland remark that the sheep which had received a comparatively large dose were quite sick when subsequently inoculated with a virulent culture, and the immunity acquired was less reliable than that obtained by

Pasteur's method with two vaccines of different degrees of attenuation.

In an investigation made by Hankin, in the laboratory of Professor Koch at the Hygienic Institute of Berlin, the results of which are given in a preliminary account published in the *British Medical Journal* (October 12, 1889), the important fact was ascertained that immunity may be produced in susceptible animals by inoculating them with an "albumose" isolated from anthrax cultures. Hankin gives the following account of his method of obtaining this immunizing proteid from anthrax cultures:

"In the course of my process of preparation it is precipitated from its solution by the addition of a large bulk of absolute alcohol, and well washed in this liquid to free it from ptomaïnes; it is well known that all such substances are soluble in alcohol. It is then filtered off and dried; then it is redissolved and filtered through a Chamberland filter. A rough estimate of the percentage of albumose present in the clear solution thus obtained is made colorimetrically by means of the biuret reaction and a peptone solution of known strength."

"In one experiment four rabbits (Nos. 23 to 26) were inoculated subcutaneously with virulent anthrax spores. No. 26 served as a control and died in about forty hours. The other three rabbits had the albumose solution injected into the ear-vein at the same time. Nos. 24 and 25 each had about the five-millionth of their body-weight, while No. 23 had only the ten-millionth of its body-weight of albumose. No. 25 died in less than forty-eight hours, but Nos. 23 and 24 survived. Ten days later Professor Koch kindly reinoculated these two rabbits for me with very virulent anthrax from an agar-agar culture. Their temperature has remained normal since then, and they are now alive and well a fortnight after this operation. I have also succeeded in producing immunity in mice against attenuated anthrax."

In a paper published in the Proceedings of the Royal Society in 1890, Dr. Sidney Martin has given an account of his researches relating to "The Chemical Products of the Growth of Bacillus Anthracis, and their Physiological Action." In his experiments the cultures were maintained for from ten to fifteen days, and the bacilli were then removed by filtering through a Chamberland filter. The filtrate was found to contain:

"1. Proto-albumose, deutero-albumose, and a trace of peptone, all with the same chemical reactions as the similar bodies formed in peptic digestion. 2. An alkaloid. 3. Small quantities of leucin or tyrosin. The chief characteristic of the proto- and deutero-albumose obtained from anthrax cultures was found to be their strong alkalinity in solution. This was not removed by prolonged dialysis or by washing in alcohol, chloroform, benzene or ether. These proteids are precipitated in an alkaline condition by saturation with NaCl (proto-albumose) or $(NH_4)2SO_4$."

The alkaloid found was soluble in water or in absolute alcohol, was strongly alkaline in solution, and readily formed salts with acids. It was slightly volatile and lost its poisonous properties to a great extent when exposed to the air for some time. A mixture of the two albumoses was toxic, and when injected into mice in small amounts caused a local subcutaneous œdema ending in recovery. Larger doses caused more extensive œdema and death. A fatal dose for a mouse weighing 22 gm. was 0.3 gm. Boiling for a short time diminished the toxicity of these proteids without completely destroying it. The alkaloid produced similar symptoms when injected into mice but more promptly and in a smaller dose—0.1 to 0.15 gm. killed a mouse weighing 22 gm. in two or three

hours. Hankin and Westbrook have more recently (1892) made researches with reference to the proteids present in anthrax cultures. To obtain an immunizing albumose they cultivated the bacillus at 20° C. in flesh-extract solution (1 to 1,000) to which fibrin was added. At the end of eight days a considerable precipitate was obtained by means of ammonium sulphate. This was placed in a dialyzer in running water at 42° to 45° C.; then precipitated by alcohol and dissolved in a small quantity of water (30 c.c.)—500 c.c. of flesh-extract treated in this way gave only 0.44 gm. of albumose. Experiments on mice gave some evidence of the immunizing action of this albumose, but the results were apparently not so definite as those previously reported by Hankin (p. 89). Nor are the experiments of Petermann, who followed Hankin's method (1892), more satisfactory. Arloing obtained more favorable results by using culture liquids from which the bacilli had been removed by sedimentation. A considerable precipitate was obtained when alcohol was added to the culture liquid, but it was found that this precipitate had no immunizing effect. On the contrary, there remained in solution an immunizing substance. This was obtained in a concentrated form by evaporating at 50° C. in a partial vacuum. Experiments upon lambs showed the protective power of this extract, and of the culture liquids before treatment when injected in considerable quantity.

In a paper published in the *Fortschritte der Medicin*, Wysokowicz gives a *résumé* of the results obtained in Russia in protective inoculations made up to date of publication (January, 1889). According to the author named, Professor Cenkowski, who had made himself familiar with Pasteur's method while on a visit to Paris,

was the first to employ it in Russia (1883). But he found its application to be attended with some difficulties. The cultures attenuated as directed by Pasteur at 42° to 43° C. "showed a very different degree of virulence in different experiments, and their virulence was also changed by keeping." Experiments were therefore made with a view to securing a more satisfactory vaccine. In an experiment made in 1885, 1,333 sheep were inoculated; of these 21 died from the first inoculation and 4 from the second (1.86 per cent.). Subsequently better results were obtained, and up to the end of 1888, 20,310 sheep had been inoculated, with an average mortality of 0.87 per cent. as a result of the inoculations.

Professor Cenkowski found that greater losses occurred when the inoculations were made in midsummer or midwinter than when they were made in the spring or autumn. The losses from anthrax diminished among the flocks in which the protective inoculations were practised in proportion to the number of sheep inoculated, falling from 8.3 per cent. in 1884, the year before the inoculations were commenced, to 0.13 per cent. in 1888. The author of the paper states that in some parts of Russia the annual loss among the sheep from anthrax is as high as thirty-three per cent.

The reliability of the protective inoculations was tested by a Commission, to which Wysokowicz belonged. Fifty sheep which had been inoculated from two to four months previously were infected with virulent anthrax material. Of these one only died. Later, twenty sheep which had been inoculated thirteen months before were inoculated with virulent material. Of these two died. These favorable results are ascribed by Wysokowicz to the improved method of attenuating anthrax virus

adopted by Professor Cenkowski. As a first vaccine he employed a culture which was stronger than that of Pasteur, and which killed mice and caused the death of one-third of the Zieselmäuse (Spermophilus citillus) inoculated. He used as a vaccine an attenuated culture which had been carried through a series of the animals last mentioned. His vaccine, consisting of a bouillon culture from a drop of blood of the animal, was preserved by the addition of two parts of a thirty per cent. solution of pure glycerine to one part of the culture.

For inoculating a sheep of average size he used 0.1 to 0.2 c.c. of this first vaccine, for a larger animal from 0.3 to 0.5 c.c. This second inoculation was made twelve days after the first, with a virus which killed three-fourths of the Zieselmäuse and from one-third to one-half of the rabbits inoculated with it. Numerous experiments convinced Cenkowski that no change occurred in the virulence of his different vaccines when they were carried through a series of mice or of earless marmots (Zieselmäuse).

Hess reports that the anthrax inoculations made by Chauveau's method in the Canton Bern, during the years 1886, 1887, and 1888, were not attended with any losses either from the inoculations or from subsequent attacks of anthrax among the inoculated animals (cattle?). In all two hundred and fifty-three animals were inoculated during the three years specified.

Hutyra (1890) has reported upon anthrax inoculations by Pasteur's method, as carried out under the regulations of the Government in 1889. The number of horses inoculated was 130, 2 of which died of anthrax at a later date —not as a result of the inoculation. This gives a percentage of loss of 1.35, which is much below the usual rate

without protective inoculations. Three thousand two hundred and seventy-nine cattle, belonging to thirty-two different estates, were inoculated. Of these 11 died from anthrax, and 2 of these as a result of the first inoculation. Deducting these 2 the loss was 0.27 per cent., whereas in former years the losses in the same herds had been from six to twelve per cent. Twenty-two thousand seven hundred and sixty-seven sheep were inoculated on twenty-three different estates. One hundred and sixty-two of these died from the first inoculation and 59 within twelve days after the second inoculation. In the course of the year 432 of the inoculated animals died from anthrax—in all a loss of 2.18 per cent. In the absence of protective inoculations the annual loss in these flocks had been about ten per cent. It was found that lambs four months old could be inoculated with the same dose as the older sheep, and without any greater loss as a result of the operation.

The result of anthrax inoculations made in France by Pasteur's method during the past twelve years have recently (1894) been summarized by Chamberland. The veterinarians who made the inoculations were each year called upon to answer the following questions: 1. Number of animals inoculated. 2. Number of deaths from first inoculation. 3. Number of animals dying within twelve days after second inoculation. 4. Number of animals dying of anthrax within a year after protective inoculations. 5. The yearly average loss before inoculations were practised. The total number of animals inoculated during the period to which this report refers was 1,788,677 sheep and 200,962 cattle. The average annual loss before these protective inoculations were practised is said to have been about ten per cent. for sheep

and five per cent. for cattle. The total mortality from this disease among inoculated animals, including that resulting from the inoculations, was 0.94 per cent. for sheep and 0.34 per cent. for cattle. Chamberland estimates that the total saving as a result of the inoculations practised has been 5,000,000 francs for sheep and 2,000,000 francs for cattle.

Podmolinoff gives the following summary of results obtained in 1892 and 1893 in the "Government of Cherson" (Austria): Number of sheep inoculated, 67,176; loss, 294, = 0.43 per cent. Number of horses inoculated, 1,452; loss, 8. Number of cattle inoculated, 3,652; loss, 2. The conclusion is reached that Pasteur's method of inoculation affords an immunity against infection with virulent anthrax bacilli in greater amounts than could ever occur under natural conditions.

Serum-therapy.

The Japanese bacteriologists, Ogata and Jasuhara, published in 1890 their paper relating to the modification of virulence in anthrax bacilli by cultivation in the blood-serum of immune animals and the therapeutic use of blood-serum from the frog or dog for the cure of anthrax in susceptible animals after infection. It was found that an anthrax culture which killed mice, when grown upon blood-serum from immune animals, such as the frog, the rat, or the dog, became attenuated to such an extent that when inoculated into mice a fatal result no longer occurred. But more surprising was the announcement that when mice received a drop of frog's blood, or half a drop of dog's blood, some time before (as much as seventy-two hours) or shortly after (five hours) infection

with anthrax bacilli, they all recovered and subsequently proved to be immune. When the blood of the frog, dog, or rat was subjected to a temperature of 45° C. for an hour it no longer had any curative or immunizing action. Similar experiments are said to have been made upon rabbits and guinea-pigs with the same result. According to the authors mentioned the proportion of dog's blood required to protect a mouse from the fatal effects of an anthrax inoculation is about one part to eight hundred parts of body-weight. But these remarkable results reported by the Japanese investigators have not been confirmed by subsequent researches. Several Italian bacteriologists, who repeated the experiments, in 1891, failed to cure infected animals with considerably larger doses of serum from immune animals. Thus Pane found that the blood-serum of dogs and pigeons did not preserve guinea-pigs from anthrax infection. Bergonzini injected blood-serum of the dog into the peritoneal cavity of guinea-pigs in doses of 3.5 to 17 c.c. without preserving them from fatal infection, no matter whether the serum was injected before or after or at the same time as the anthrax virus. Serafini and Enriquez (1891) also failed entirely to prevent a fatal result after anthrax infection by injecting serum from immune animals into the animal being experimented upon. Their experiments were made upon 22 rabbits, 43 guinea-pigs, 3 white mice, and 38 gray mice, all of which died of anthrax. The quantity of serum or blood used varied from one drop to 25 c.c., and three rabbits received several doses of dog serum of 40 c.c. each. Injections were made subcutaneously, or into the peritoneal cavity, and in some instances into the circulation through a vein. The injection of a mixture of 1 öse of an anthrax culture with 5

c.c. of blood-serum caused the death of the inoculated animal.

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II.

CHICKEN CHOLERA.

Pasteur's researches with reference to the etiology of the disease known in France as *choléra des poules*, first led him to the discovery that a virulent culture of a pathogenic bacterium may become "attenuated" by certain agencies, and that immunity may be conferred upon susceptible animals by inoculating them with such attenuated culture. We now know that his microbe of fowl cholera is a widely distributed bacillus, which is frequently encountered in putrefying material, and that it is also extremely fatal to pigeons, pheasants, sparrows, rabbits, and mice. Also that the same, or nearly allied species, may produce an infectious disease of swine (*Schweineseuche*), of cattle (*Rinderseuche*), and of deer (*Wildseuche*).

Subcutaneous injection of a minute quantity of a virulent culture usually kills chickens within forty-eight hours. Some time before death the fowl falls into a somnolent condition, and, with drooping wings and ruffled feathers, remains standing in one place until it dies. Infection may also occur from the ingestion of food moistened with a culture of the bacillus, or soiled with the discharges from the bowels of other infected fowls. At the autopsy the mucous membrane of the small intestine is found to be inflamed and studded with small hemorrhagic foci, as are also the serous membranes; the

spleen is notably enlarged. The bacilli are found in great numbers in the blood, in the various organs, and in the contents of the intestine. In rabbits death commonly occurs in from sixteen to twenty hours, and is often preceded by convulsions. The temperature is elevated at first, but shortly before death it is reduced below the normal. The post-mortem appearances are: Swelling of the spleen and lymphatic glands; ecchymoses or diffuse hemorrhagic infiltrations of the mucous membranes of the digestive and respiratory passages, and in the muscles; and at the point of inoculation a slight amount of inflammatory œdema. The bacilli are found in considerable numbers in the blood within the vessels, or in that which has escaped into the tissues by the rupture of small veins. They are not, however, so numerous as in some other forms of septicæmia—*e.g.*, anthrax, mouse septicæmia—when an examination is made immediately after death; later, the number may be greatly increased as a result of post-mortem multiplication within the vessels. The rabbit is so extremely susceptible to infection by this bacillus that inoculation in the cornea by a slight superficial wound usually gives rise to general infection and death. This animal may also be infected by the ingestion of food contaminated with a culture of the bacillus. It is by this means that Pasteur proposed to destroy the rabbits in Australia, which have multiplied in that country to such an extent as to constitute a veritable pest. Both in fowls and in rabbits the disease may, under certain circumstances, run a more protracted course—*e.g.*, when they are inoculated with a small quantity of an attenuated culture. In less susceptible animals—guinea-pigs, sheep, dogs, horses—a local abscess, without general infection, may result from the subcutaneous injection of

the bacillus; but these animals are not entirely immune. In the infectious maladies of swine, cattle, deer, and other large animals, to which reference has been made, and which are believed to be due to the same bacillus, the symptoms and pathological appearances do not entirely correspond with those in the rabbit or the fowl; but the bacillus as obtained from the blood of such animals corresponds in its morphological and biological characters with Pasteur's microbe of fowl cholera, and Koch's bacillus of rabbit septicæmia, and pure cultures from the various sources mentioned, are equally fatal to rabbits and to fowls. In the larger animals pulmonary and intestinal lesions are developed, and in swine, a diffused red color of the skin, similar to that observed in the disease known in Germany as *Schweinerothlauf* (Fr. *rouget*), is sometimes seen.

According to Baumgarten bacilli from *Wildseuche* or from *Rinderseuche* inoculated into swine give rise to fatal *Schweineseuche*, and bacilli from any of these forms of disease, when inoculated into pigeons, produce characteristic fowl cholera; but the bacillus as obtained from *Schweineseuche* or *Wildseuche* is not fatal to chickens, and the bacillus from *Schweineseuche* is fatal to guinea-pigs, which have but slight susceptibility to the bacillus of rabbit septicæmia. Notwithstanding these differences he agrees with Hueppe in the view that the bacilli from the various sources mentioned are specifically identical; although evidently, if this view is adopted, we must admit that varieties exist, which differ somewhat in their pathogenic power.

In the writer's "Manual of Bacteriology" this bacillus is described under the name *Bacillus septicæmiæ hemorrhagicæ*, first proposed for it by Hueppe. In the present

chapter we shall give an account of the experimental evidence relating to protective inoculations and serum therapy in various animals, with the different varieties of the bacillus in question which have been encountered.

It seems probable that the same bacillus was the cause of the fatal form of septicæmia studied by Davaine, and which resulted from the inoculation of susceptible animals with putrefying blood. These experiments by the distinguished French physician constitute an important part of the pioneer work in this field of research. They were commenced in 1868, and are published in the *Bulletin of the Academy of Medicine* (*séance* of February 18, 1879).

Davaine, in the paper referred to, calls attention to the fact, developed by his experiments, that there is a great difference in the resisting power of different animals to the form of septicæmia which had been the subject of his investigations. Thus the rabbit succumbed when inoculated with a millionth part of a drop of blood, while guinea-pigs and dogs remained unaffected by such small doses. With reference to the specific cause of the form of septicæmia discovered by him, Davaine says:

"The virus is one of the bacteria of putrefaction. I say 'one of the bacteria' because there is reason to believe that there are among these minute organisms numerous species which do not all develop at the same time when they are present in various media."

Davaine also discovered the fact that infection depends, within certain limits, upon the quantity of bacteria introduced into the tissues. He says:

"This question of quantity was manifest in our experiments. Not only did it vary in different species, the

rabbit and the dog for example, but it may vary in the same species."

The identity of "Davaine's septicæmia" with Pasteur's *choléra des poules* is made still more probable by the experimental evidence offered by Toussaint in a communication to the French Academy of Sciences, made by M. Bouley at the *séance* of July 25, 1881. In this communication Toussaint says:

"Three years ago, July 8, 1878, I had the honor to present to the Academy an account of a malady due to microbes, which I identified with that studied by Davaine in 1864 and 1865, and which he differentiated from anthrax, for which it had been mistaken by Leplat and Jaillard.

"In the month of December, 1878, I made acquaintance with fowl cholera, and already, in my thoughts, I identified this disease with that which I had observed in my experiments made early in the year. The microbes of the two diseases resembled each other perfectly and behaved the same when inoculated in rabbits. I had, even in 1879, sent to M. Bouley two notes, in which I called attention to the analogies which exist between the parasites of the two diseases and the lesions which they determine, not only in the rabbit but also in pigeons and fowls.

"The experiments of the same kind made at the end of 1879 and in 1880 caused me to insert the note published on page 301, vol. xci., of the *Comptes-rendus*, under the title of: 'Identity of Acute Experimental Septicæmia and Fowl Cholera.' I gave a *résumé* in this note of five series of experiments which had demonstrated to me that inoculations of the microbe of septicæmia give rise to the manifestations of fowl cholera. These results have recently been confirmed by additional facts."

Toussaint closes his paper by some remarks upon the origin of epidemics of fowl cholera which we quote be-

cause we believe that the additions made to our knowledge of the microbe which causes this disease give support to the views advanced by him in 1881:

"The causes which determine epidemics of fowl cholera are yet unknown. It has been supposed that putrefactive substances may give rise to them, and this has led to the recommendation of cleanliness and disinfection for their prevention. The microbe which kills the first fowl in an epidemic certainly came from some anterior generation which had killed others. But how was it perpetuated? Do not the facts which demonstrate the development of septicæmia from material undergoing putrefaction throw some light on the question of etiology? Is it not probable that the fowls find the conditions of infection with cholera in the presence of organic matter undergoing putrefaction, which may serve as a culture medium for the germs of septicæmia which are in suspension in the air together with the ordinary germs of putrefaction?"

Pasteur's first communication relating to the etiology of fowl cholera was made to the French Academy at the *séance* of February 9, 1880. In this communication he calls attention to the fact that when fowls are fed with bread or meat soiled with a small quantity of a culture of the microbe of fowl cholera they become infected and their discharges contain the bacillus in large numbers, a fact which readily accounts for the spread of the disease in a poultry-yard when a case occurs.

In the same communication Pasteur records his observation that "by a certain change in the method of cultivation the infectious microbe may be caused to have a diminished virulence." Also the fact that fowls inoculated with this "attenuated" virus recover and are subsequently immune against infection by the most virulent microbes. In concluding this communication Pasteur says:

"It appears to be superfluous to point out the principal result of the facts which I have had the honor to present to the Academy. There are two, however, which it may be useful to mention. These are, first, the hope of obtaining artificial cultures of all kinds of virus; second, the idea of seeking for virus vaccines of the virulent maladies which have devastated so often, and still devastate, the human race, and which are such a scourge to that branch of agriculture which relates to the breeding of domestic animals."

In his communication of October 26, 1880, Pasteur gives his reasons for concluding that attenuation of virulence is due to the action upon the microbe of atmospheric oxygen. He infers this from the fact, demonstrated by experiment, that when cultures are placed in hermetically sealed tubes, from which the oxygen present is soon exhausted by the growth of the microbe, they do not become attenuated in virulence; whereas cultures which are freely exposed to the air gradually become attenuated. Pasteur sees in this an important fact bearing upon the explanation of the natural extinction of epidemics. He says:

"May we not suppose, then, that it is to this influence that we must attribute, in the present as in the past, the limitation of great epidemics."

In his communication to the French Academy, made on February 28, 1881, Pasteur treats of the attenuation of virulence by the method above referred to and by the method of Toussaint, and also of the re-establishment of the virulence of attenuated cultures. He says:

"The secret of the return to virulence rests solely, at present, upon successive cultures in the bodies of certain animals."

Thus he had found by experiment that the anthrax bacillus might be so attenuated that it was harmless for grown guinea-pigs, or even for guinea-pigs a month or a week old, but it would still kill guinea-pigs just born—a day old. By inoculating an older pig with the blood of this one, and so on, the virulence was gradually augmented, until finally a virus might be obtained which would kill adult animals, and even sheep. In the same way the attenuated microbe of fowl cholera could be restored to virulence by first inoculating small birds, such as sparrows or canaries.

Applying these facts, demonstrated by his experiments, to the explanation of the origin of epidemics, Pasteur says:

"I finished my communication on October 26th by calling attention to the attenuation of viruses by exposure to the air as being probably one of the factors in the extinction of great epidemics. The facts presented in this paper, in their turn, may serve to explain the so-called 'spontaneous development' of these scourges.

"An epidemic which has been extinguished by the attenuation of its virus may be reborn by the reinforcement of this virus under certain influences. The accounts which I have read of the spontaneous appearance of the plague appear to me to offer examples of this; for example, the plague at Benghazi, in 1856–58, the outbreak of which could not be traced. The plague is a virulent malady which belongs to certain countries. In all of these countries its attenuated virus ought to exist, ready to resume its active form when conditions as to climate, famine, and distress again occur. There are other virulent maladies which appear 'spontaneously' in all countries; such as camp typhoid. Without doubt the germs of the microbes which cause these last-mentioned maladies are everywhere distributed. Man carries them

upon him or in his intestinal canal without great damage, but ready to become dangerous, when, owing to constipation or to successive development upon the surface of wounds, in bodies enfeebled or otherwise, their virulence is progressively reinforced."

We believe that the more complete our knowledge relating to the origin and extinction of epidemics, of the kind referred to by Pasteur, becomes, the more apparent will be the value of his inductions and the clearness of his scientific foresight.

Toussaint, on July 25, 1881, reported the results of his experiments upon protecting fowls by a "new method of vaccination." This consisted in inoculating them with the blood of a rabbit which had recently died from septicæmia produced by the same microbe. As a result of such inoculations the fowls had slight local lesions at the point of inoculation, and soon recovered. They were subsequently found to be immune. Cultures from the blood of a septicæmic rabbit were found to act in the same way. When the culture had been passed through a pigeon, and had then killed a fowl, according to Toussaint, it preserved its virulence when subsequently passed through the rabbit.

Salmon, in the "Report of the Commissioner of Agriculture" for 1881 and 1882, gives an account of his experiments in producing immunity by the use of a diluted virus. He says:

"The experiments of Chauveau, taken with my own, indicate that this method is capable of generalization to the same extent as that discovered by Pasteur; while the ease and quickness with which the vaccine is prepared, the certainty of effects, the economy of material, and the more perfect protection are points which would appear

to make it decidedly superior. Wherever the cholera of fowls is raging a standard cultivation may be made and the vaccine obtained within twenty-four hours; a single drop of such a cultivation will vaccinate ten, twenty, or even forty thousand fowls, and within three weeks of the commencement of the work the most susceptible of our fowls are insusceptible to inoculations with the strongest virus. And this, without any sickness, or even local necroses, which Pasteur describes as following vaccinations with his attenuated virus."

In discussing the practical value of this method Salmon estimates the cost as trifling—"not more than half a day's time of one man for one hundred fowls, even if three inoculations were made."

In a paper on protective inoculations against fowl cholera, by Kitt, in the *Deutsche Zeitschrift für Thiermedicin* (December 20, 1886), the conclusion is reached that these inoculations undoubtedly protect the fowls from infection either in the natural way or by inoculations with virulent material. But Kitt doubts the practical utility of the method for the arrest of epidemics of this disease in the poultry-yard; and, as we think, with justice, prefers to depend upon cleanliness, disinfection, and prompt removal of infected fowls. As he points out, a considerable time is required to produce complete immunity, and two inoculations are often insufficient. Pasteur had previously reported that a third inoculation is usually required. But the infection spreads so rapidly when an epidemic is developed in a poultry-yard that a large proportion of the fowls would be likely to perish before the protective inoculations could be carried out. Another objection is that when inoculated in the breast muscle the value of the fowl for the table is reduced, and when inoculated in the wing an unpleasant-looking scab is left

at the point of inoculation. The cost in material and time required to carry out the three successive inoculations is also an objection to the practical application of the method. Moreover, the excreta of the inoculated fowls contain the pathogenic microbe, and it would evidently be unwise to practise inoculations in poultry-yards not already infected. Kitt states, also, that he has always succeeded in stamping out the disease very promptly by the other measures referred to—disinfection, cleanliness, separation of all fowls which show any indications of being infected.

In a more recent paper (1893) Kitt reports his success in conferring immunity upon fowls by a new method, which is, however, rather of scientific interest than of practical value. He first experimented to see whether the blood-serum or tissue juices of immune fowls would give immunity against cholera to other fowls, and obtained a successful result. He was not, however, able to produce immunity in pigeons or in rabbits by the same method. He next undertook to determine whether the immunizing substance was present in the eggs of fowls which had an immunity as a result of protective inoculations. The albumin and yolk of the egg, in doses of 5 to 10 c.c., was injected into the breast of fowls, and at the end of ten days a second inoculation of the same kind was made. Six days after the second inoculation the fowls (five) and a control hen were inoculated with virulent blood from a pigeon, and at the same time fed with the chopped-up flesh and liver of a pigeon just dead from fowl cholera. The control hen died on the following day from typical cholera, the others remained in perfect health.

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III.

CHOLERA.

The spirillum discovered by Koch in 1884 is now pretty generally recognized as the specific cause of Asiatic cholera. But recent researches indicate that there are numerous pathogenic varieties of this spirillum, and show that either an attenuated cholera spirillum or a closely allied saprophyte is not infrequently found in the water of rivers in various parts of Europe. As this spirillum is found in the intestine of cholera patients, and not in the blood, it is evident that its pathogenic action depends upon the chemical products developed during its growth, and this inference is fully justified by the results of experiments upon the lower animals. These chemical products have been studied by Brieger, Pfeiffer, Scholl, Gamaleïa, Westbrook, and others.

Brieger (1887) succeeded in isolating several toxic ptomaïnes from cultures of the cholera spirillum, some of which had previously been obtained from other sources —cadaverin, putrescin, creatinin, methyl-guyanidin. In addition to these he obtained two toxic substances not previously known. One of these is a diamin, resembling trimethyldiamin; it gave rise to cramps and muscular tremor in inoculated animals. The other poison reduced the frequency of the heart's action and the temperature of the body in the animals subjected to experiment. In more recent researches made by Brieger and Fränkel

(1890), a toxalbumin was obtained from cholera cultures which, when injected subcutaneously into guinea-pigs caused their death in two or three days, but had no effect upon rabbits.

Pfeiffer has more recently (1892) published his extended researches relating to the cholera poison. He finds that recent aërobic cultures of the cholera spirillum contain a specific toxic substance which is fatal to guinea-pigs in extremely small doses. This substance stands in close relation to the bacterial cells, and is perhaps an integral part of the same. The spirilla may be killed by chloroform, thymol, or by desiccation, without apparent injury to the toxic potency of this substance. It is destroyed, however, by absolute alcohol, by concentrated solutions of neutral salts, and by the boiling temperature, and secondary toxic products are formed which have a similar pathogenic action but are from ten to twenty times less potent. Similar toxic products were obtained by Pfeiffer from cultures of the Finkler-Prior spirillum and from spirillum Metchnikovi.

Scholl (1890) took advantage of the fact, previously demonstrated by Hueppe, that cultures of the cholera spirillum in egg-albumin, in the absence of oxygen, are more toxic than ordinary bouillon cultures. Cultures were made by Hueppe's method in hen's eggs. No poisonous ptomaïnes were found, but two toxic albuminous substances were obtained. The albuminous liquid from the egg cultures was dropped into ten times its volume of absolute alcohol, which caused a white precipitate, a portion of which sank to the bottom while another portion floated on the surface. The portion which floated was easily dissolved in a very dilute solution of potash and could be precipitated from this solution by the careful addition of acetic

acid, but dissolved in an excess of this acid. It dissolved also in a seven per cent. salt solution, but was precipitated by a saturated solution. It gave the biuret and xanthoprotein reaction. This substance proved to be very poisonous. It killed guinea-pigs within twenty minutes when a few cubic centimetres of the alkaline solution—potash—was injected into the cavity of the abdomen. Scholl calls this substance cholera-toxo-globulin. The precipitate which fell to the bottom of the receptacle was washed with alcohol, then digested with water for twenty minutes at 40° C. Very little was apparently dissolved out by this procedure, but this little proved to be very toxic. In from one to three minutes after the injection of a few cubic centimetres of the solution into the peritoneal cavity of a guinea-pig the animal died. This aqueous solution gave the biuret and xanthoprotein reaction; it was precipitated by mercuric chloride, nitrate of mercury, and tannin, but not by a saturated solution of ammonium sulphate or acetic acid. This substance Scholl calls cholera-toxo-pepton. The toxic action of these substances is destroyed by a temperature of 100° C., maintained for half an hour, or by 40° to 45° C., maintained for twenty-four hours. But at ordinary temperatures they retain their toxic action for several weeks.

Gruber (1892) has also obtained a toxic albuminous precipitate by allowing egg cultures to fall into alcohol, drying the precipitate, and then extracting it with water.

Gamaleïa (1893) has obtained a toxin which produces the typical phenomena of cholera, which, according to him, is closely associated with the bacterial cells, but can be extracted by a soda solution or by heating to 55° to 60° C. The conclusion is reached that it is a nucleo-albumin analogous to the toxalbumins of tetanus and of

diphtheria. It is precipitated by alcohol, acids, and by magnesium sulphate.

Finally, Westbrook, in a still more recent research (1894), arrives at the conclusion that the cholera spirillum produces various toxic proteids which in small amounts produce immunity in susceptible animals, and the production of which depends to a certain extent upon the culture medium; or that its toxin is a substance of constant chemical composition which is mixed with various albuminous substances, either contained in the culture medium or developed in the culture. Duclaux is of the opinion that the last supposition is correct, and that the so-called toxalbumins are not bodies of definite chemical composition, but mixtures of toxins and albuminous substances.

Experiments made upon the lower animals show that the introduction of these cholera toxins into the body of a susceptible animal, either with or without the living cholera spirillum, results in the establishing of a certain degree of immunity against the toxic action of cholera cultures. And there is good reason to believe that a non-fatal attack of cholera in man gives the individual a relative immunity from subsequent attacks, for some time at least. This has led to extended experiments with reference to the possibility of producing a similar immunity in man by means of protective inoculations. The experiments bearing upon this point which have been made upon the lower animals will first engage our attention.

Hueppe (1887) first demonstrated the fact that injections of a small amount of a cholera culture into the peritoneal cavity of a guinea-pig is fatal to these animals.

In the following year (1888) Gamaleïa reported his success in infecting guinea-pigs by subcutaneous injections

of blood from an infected pigeon. He found that by successive inoculations in pigeons a considerable increase in virulence is established, and, that while guinea-pigs were not fatally infected by subcutaneous inoculations with ordinary cultures, they invariably died when inoculated with the more virulent culture in the blood of an infected pigeon. Also, that when guinea-pigs were inoculated with ordinary cultures, or with cultures sterilized by heat, they were subsequently immune, and resisted inoculations with the most virulent material. In the same year the author referred to announced the discovery of a spirillum which closely resembles the cholera spirillum—his "Vibrio Metchnikovi." This was obtained from the intestinal contents of fowls suffering from a fatal infectious malady (in Odessa). According to Gamaleïa chickens and pigeons which have survived an inoculation with a culture of this spirillum are subsequently immune against the pathogenic action of the cholera spirillum, and *vice versa*. In subsequent communications Gamaleïa reported that sterilized cultures of his "Vibrio Metchnikovi" (sterilized by heat at 120° C.) were very pathogenic for rabbits, fowls, pigeons, and even for dogs and sheep. The rabbit proved to be the most susceptible animal, and succumbed to doses of 4 c.c. in from twelve to twenty hours. Doses of 1 c.c. per 100 gm. of body-weight caused a temporary indisposition followed by immunity. Pigeons were made immune by larger doses.

The researches of Pfeiffer (1889) confirmed those of Gamaleïa as to the fact that pigeons and guinea-pigs could be made immune against Vibrio Metchnikovi by the injection of sterilized cultures. But guinea-pigs which had been immunized against this pathogenic spirillum succumbed to cholera infection; and, on the other hand, ani-

mals which had been treated in various ways with a cholera culture died without exception when infected with Vibrio Metchnikovi. The conclusion is therefore reached that the two pathogenic spirilla are distinct species, although very similar in many respects.

Brieger and Wassermann (1892) have reported the results of experiments with the cholera spirillum cultivated in thymus bouillon. After twenty-four hours development in this medium the cultures were sterilized by heat (55° C. for fifteen minutes) and placed in an ice-chest for twenty-four hours. Four c.c. of this fluid injected daily for four days into the peritoneal cavity of a guinea-pig made it immune to the cholera spirillum in doses three times as large as were required to kill an animal not so treated. This immunity lasted for two months. Fedoroff (1892) obtained similar results by the subcutaneous injection of sterilized cultures in doses of 1 c.c., in guinea-pigs. His cultures in thymus bouillon were kept for from seven to ten days at 37° C., then sterilized by heating for fifteen minutes at 65° C., then allowed to stand in a dark room for twenty-four hours, and finally mixed with an equal volume of glycerine.

Ketscher (1892) has obtained evidence that the immunizing substance in animals which have received protective inoculations is contained in the milk of females thus treated. Three goats received subcutaneous inoculations of virulent cholera cultures, and also injections into a vein and into the peritoneal cavity. The milk of these goats was injected into the peritoneal cavity of rabbits; these proved to be immune when subsequently lethal doses of a virulent cholera culture were injected into the peritoneal cavity.

According to Gamaleïa (1892), dogs are very suscepti-

ble to infection with cholera spirilla, and present symptoms closely resembling those of cholera in man. They may also be easily immunized against the pathogenic action of cholera cultures.

Gruber and Wiener (1892) have also found that susceptible animals are easily immunized against cholera infection either by inoculation with small doses, with attenuated cultures, or with larger quantities of sterilized cultures. Haffkine (1892) also reports his success in immunizing guinea-pigs and pigeons.

Pawlowsky (1893) claims to have obtained from the blood of animals having an acquired immunity against cholera an antitoxin in the form of an amorphous powder; and Lazarus (1892) reports that the blood of man, after recovery from an attack of cholera, has the property of protecting guinea-pigs from fatal infection when injected, in very small amount, into the peritoneal cavity. Issaeff (1894) in an extended series of experiments was not able to entirely confirm the results reported by Lazarus. In a summary of results obtained in his own experiments he says:

"1. The intraperitoneal or subcutaneous injection of blood-serum from normal individuals [that is, persons who have not suffered an attack of cholera], and also of various acids, alkalies, and neutral liquids, gives to guinea-pigs a certain resistance against intraperitoneal cholera infection. This resistance, however, is feeble and temporary, and cannot be considered as identical with the true immunity which results from vaccination with the products of the cholera bacteria.

"2. Guinea-pigs vaccinated against cholera have no immunity against the toxins of the cholera vibrio, notwithstanding their high degree of insusceptibility to infection with cultures containing the living vibrio. The

blood of immunized guinea-pigs does not possess antitoxic properties. The maximum dose of cholera toxins which immune guinea-pigs can withstand is not greater than that which control animals withstand.

"3. The blood of guinea-pigs carefully immunized against cholera possesses specific and very pronounced immunizing, and, in a certain sense, curative powers.

"4. The blood of cholera convalescents possesses similar specific and curative powers. This property is first developed about the end of the third week after the attack, and disappears completely at the end of two or three months."

In a series of experiments made by Pfeiffer and Issaeff the results obtained, as stated by Pfeiffer in a subsequent communication, were as follows:

"In my research with Issaeff 'upon the explanation of cholera immunity' I proved that the serum of animals which have an active acquired immunity against cholera only has a specific action upon this particular species of vibrio, and as regards other species of bacteria does not differ in its action from the blood-serum of normal animals. We also showed that this specific influence in respect to the intraperitoneal cholera-infection of guinea-pigs was due exclusively to bactericidal processes which in some way were induced by the serum of immune animals."

The view of Pfeiffer, founded upon his experimental results, is that the destruction of the living cholera spirilla, which quickly takes place in the peritoneal cavity of the guinea-pig, when at the same time a minute quantity of serum from an immune animal is introduced, is not directly due to the bactericidal action of this serum, but that, in some way, it gives rise to a specific bactericidal action in the exudate which is found in the peritoneal

cavity as a result of such injections. His experiments also lead him to the conclusion that this is accomplished quite independently of phagocytosis.

The brief review of experimental researches relating to cholera immunity which we have made shows that, while there is a general agreement as to the possibility of producing immunity in susceptible animals, there is considerable difference of opinion as to the true explanation of this immunity. The supposition that it is due to an antitoxin which has the power of neutralizing the toxic products of the cholera spirillum does not receive any support from the most recent investigations—those of Pfeiffer and Issaeff—which, on the contrary, seem to establish the fact that this immunity depends upon an increased bactericidal activity of the blood-serum of immune animals. A very curious fact developed by the researches of the bacteriologists last named is that—

"The cholera-serum, which in the peritoneal cavity of guinea-pigs acted only upon the cholera bacteria, and behaved toward other vibrios exactly like the serum of normal animals, in a test-tube killed all four species of vibrios with equal rapidity."

Unfortunately the evidence relating to the value of protective inoculations in man, although supported by the evidence already referred to as regards the lower animals, is, to a considerable extent, unsatisfactory, owing to the difficulty of applying scientific methods to experiments of this kind. The evidence, however, is in favor of the view that a certain degree of protection is afforded by the subcutaneous injection of cholera cultures. Such protective inoculations could not be expected to confer an absolute immunity, inasmuch as the immunity result-

ing from a single attack has only a relative value, and is probably not of long duration.

We quote from Shakespeare's "Report on Cholera in Europe and India, 1890," the following paragraphs relating to immunity as a result of an attack of cholera:

"IMMUNITY AFTER AN ATTACK OF CHOLERA—EXPERIENCE IN FRANCE, 1884.

"The Academy of Medicine of Paris directed a circular letter of questions concerning cholera to the physicians of the localities infected by that disease in 1884, and in group L of general observations in that questonario is found the following: 'Have there been observed recurrences among the people attacked, either in a former epidemic or in the present one? Give the results of this recurrence.' In response to their questions the Academy received 184 communications, but the committee appointed to analyze them eliminated 79; for various reasons given only 104 were used for analysis. Of this number only 8 bore upon the particular question above mentioned, and it is reasonable to assume that the other 96 observers said nothing concerning this point because they had observed nothing bearing upon it. The results of this analysis may be stated as follows:

"From Castelnaudary, with a population of 10,000, we learn that there were 54 cases and 18 deaths from cholera, among which there was 1 recurrence; from Aix, with 20,257, number of cases unknown, deaths 117, among these 2 recurrences were observed, at intervals of ten and forty days; from Beseges, with 11,400 inhabitants, we learn of 124 cases and 40 deaths, among which were 2 recurrences; from Cette, with 35,000, the number of cases is not mentioned, but we learn that there were 92 deaths and 1 recurrence; from Nantes, with 124,300 inhabitants, we learn of 251 cases and 112 deaths, with 1 recurrence; from Perpignan, with 25,000 inhabitants, we hear of 325 cases and 225 deaths, and receive the indefinite statement that

there were some fatal recurrences; from Pignans, population not stated, we learn of 22 attacks and 12 deaths, with 1 recurrence; from Cadenet, with a population of 26,000, we are not informed of the number of cases, but learned that there were 20 deaths and 2 recurrences."

"IMMUNITY AFTER AN ATTACK OF CHOLERA—EXPERIENCE IN SPAIN, 1885.

"While examining cholera in Spain, the writer prepared a circular containing a series of twenty-five questions relating especially to the nature, etiology, and prophylaxis of cholera, one of which requested the physician to state whether or not, in his own personal experience, he had observed a second or a third attack of cholera during the same epidemic, and in case of a positive reply to detail the symptoms and all the circumstances surrounding it. This circular-letter was addressed to some twenty-five hundred Spanish physicians, located in the various cities, towns, and villages in that kingdom which had suffered from the epidemic. Among the large number of replies there were only 8 in which a second attack was reported, and from an examination of the details of these there was no doubt left in our mind that 6 were not genuine second attacks after a complete recovery, but were in reality relapses due to imprudences of diet or otherwise before convalescence and complete recovery had been established. Two of the 8 cases, from the details of the reports given, may have been genuine recurrent attacks of Asiatic cholera, or may have been simply seizures of cholera morbus (cholera nostras). It is well known that after an attack of Asiatic cholera the digestive apparatus is left in a damaged condition, and disorders of the intestines continue for a long time. The habits of life and the imprudences so common to the class of people most frequently suffering from Asiatic cholera in that country are such as to render them more than usually liable to suffer attacks of cholera nostras. As having an important bearing upon this suggestion, the writer

made an analysis of the vital statistics of Spain, covering the five years previous to 1885, for the purpose of learning the extent of prevalence of cholera nostras among that population, and the result of the inquiry shows that the number of deaths attributed to that disease averaged per year sixteen per every million inhabitants."

Dr. Ferrán, who practised inoculations on an extensive scale during the epidemic of 1885, in Spain, gives the following account of his method of performing these inoculations:

"1. The cholera vaccine is nothing more than a pure culture, in bouillon, of the comma bacillus. Its easy and long preservation (four to five days) allows of its transportability to great distances, taking care always to keep the flask which contains the material upright.

"2. Heat and cold do not interfere with its preservation if the vaccine is to be used in a short time. It should not, however, be kept out of doors during the warm season.

"3. The vaccine should be kept in flasks of the model of Ferrán, with a flat bottom and a short neck. The stopper, which is of rubber, fits perfectly, and is penetrated by two glass tubes, one straight and short, which does not extend below the inferior surface of the stopper, and which does not project above more than some two centimetres, is plugged with a small quantity of sterilized cotton and a superficial covering of wax. The other glass tube is longer, and extends on the lower side as far as the bottom of the flask, while its superior end is curved, and terminates in a capillary extremity, the tip of which is closed with wax.

"4. When the vaccine is to be used it is necessary to make two principal preparations for the operation. A small syringe for the hypodermic injection, and a small vessel into which it is necessary to empty the fluid from the flask are required. The syringe should have me-

tallic pistons and mountings, without mastic of any kind and without rubber. Its capacity should be 1 c.c., its needle thicker and shorter than that of ordinary use. Before beginning the vaccination the syringe must be filled two or three times with boiling water, which is aspirated and expelled through the needle. This is called sterilizing the instrument, and by this means the extraneous germs are destroyed which might be contained in it, in order to avoid the production of phlegmons and abscesses. The trouble in taking this precaution will be little. Acting thus, one may perform thousands of injections without fear of any accident. It is suggested that it is a bad custom to pass the needle through a flame in order to sterilize it, because this mode of procedure draws the temper. Another precaution that must be taken relates to the examination of the syringe before using it, in order to be well assured that the piston acts perfectly and that not a single drop of the liquid escapes by a leak in the cannula. This latter defect is sufficient to reject the instrument. If the syringe aspires air because the leather washer, which is placed at the end of the glass tube in order to facilitate its adaptation is dry, or the piston is in the same condition, it is necessary to delay a little while in order to take the syringe apart and soak it in warm water. It is convenient to keep several syringes for use, with a sufficient number of needles, when many inoculations are to be performed.

"5. The small receptacle into which the vaccine is poured in order that the syringe may be filled readily is a capsule, a cup, or some similar vessel. Before use, it should be washed and dried with extreme care, and immediately before using passed through an alcohol or Bunsen flame, in order to sterilize it.

"6. All these preparations having been made, the drop of wax which closes the capillary extremity of the long tube of the flask is removed, and at the same time also the wax covering of the cotton-stopper of the short tube, but by no means must this cotton stopper be re-

moved; a rubber tube, or the extremity of a small Richardson spray apparatus, is adjusted to the short tube. The capillary extremity of the long tube is now slightly warmed in order to soften somewhat the wax which may have been drawn into its lumen by capillarity, and air is forced into the flask, either by blowing into the rubber-tube or by working the Richardson atomizer; the air injected by pressure upon the vaccine fluid forces the latter out through the long tube with the capillary extremity, and it is collected in the cup or small sterilized vessel. This latter is then covered with white paper, which has been scorched in the flame, or with a sterilized glass plate; as often as the syringe is filled, this cover will be removed and again immediately afterward replaced.

"7. Never should the rubber stopper which closes the flask, or the cotton which plugs the short straight tube, be removed, because otherwise the germs of the external air might enter and contaminate the culture, and in this way give place to local and general accidents among the inoculated. Whenever, through the movements of transportation, the cotton plug in the short glass tube has become so wet as to impede the passage of the air which is to be forced into the flask in the act of expelling the vaccine from it, it may be removed with the point of a needle and rapidly substituted by another plug of surgical cotton which has been carbonized or salicylized. If this proceeds with cleanness and promptness, there is no danger in doing it. When the cotton, although wet, does not impede the injection of the air, it is better not to change it.

"8. After terminating the vaccination, again the capillary extremity of the curved tube is passed through the flame until the small quantity of liquid remaining in it is evaporated; it is then stopped a second time with a small drop of wax; and from the other glass tube the rubber tube which has been employed for forcing in the air is removed and another thin layer of wax is placed over the cotton plug.

"9. If in the smaller vessel or cup any of the vaccine fluid remains after the vaccination of all persons present, it is boiled, and in this manner the culture is killed, for it should not be used in another operation, because atmospheric germs might become mixed with it.

"10. The technique for the practice of the inoculation is the same as for all hypodermic injections. The most convenient region is that of the brachial triceps.

"11. The dose is 1 c.c.—or the contents of a syringe—into each arm, for individuals of all ages and conditions.

"12. Five days having elapsed, revaccination may be performed by following the same instructions."

Shakespeare, who was sent by the United States Government to Spain to investigate the results of these inoculations, reports as follows:

"And now with respect to the human inoculations: The most of these inoculations were performed in villages in the province of Valencia. The number of persons inoculated considerably exceeds thirty thousand. Much has been both said and written in Spain, France, and England concerning the results of these inoculations. The results which have been published have appeared to very strongly back up the claim of Dr. Ferrán that choleraic inoculation has the power of protecting the individual against an attack of cholera, and that the extensive practice of this inoculation among villages already invaded by the epidemic is a powerful and at the same time harmless means of bringing the epidemic to an end. This being the case, for those who were unwilling to accept the deductions to be made from the published statistics, the only way of escaping their force seemed to be by an attack upon their validity.

"The statistics of the anti-choleraic inoculations have been widely attacked. The first public onslaught upon these statistics of which the world, outside of Spain, had much knowledge, was made in the report of the French

Commission, with Dr. Brouardel at its head, which was presented to the Minister of Commerce after the return of that Commission from Spain in the summer of 1885. It is charged in that report that the results of the statistics therein reproduced are assailable on account of having been collected by physicians who were partisan supporters of Dr. Ferrán, and that they neither possessed any adequate official character, nor did they possess sufficient details. As far as I can learn, the general impression entertained throughout the world of the value of the inoculation statistics is based, in the main, upon this report of the French Commission.

"The statement of that Commission that the statistics which they had been able to obtain of the preventive inoculations of Ferrán were to a considerable degree void of any official character may be true, and perhaps it is also true that they emanated from the partisan friends of Ferrán; but it must be distinctly remembered that at that day there were practically no official statistics of this kind in the hands of anyone. The official statistics collected under the orders of the Spanish Government were gotten together at a far later date.

"Upon the appointment of the Government at Madrid of the second official Spanish Commission to investigate the Ferrán question in the provinces where the inoculations were being practised, it was ordered that official statistics of the inoculations should be collected in the usual manner; that is to say, by the customary statistical officers of the Government. This second medical Commission was also accompanied by an independent statistical commission who were charged with the duty of forming statistics of those inoculations which were expected to be witnessed by the Medical Commission in their tour of investigation, and the report to the Spanish Government of this statistical commission is based exclusively upon the official statistics which they themselves collected.

"In estimating the value of the official character and

the authority of the official statistics, which have *since the visit of the French Commission to Spain* been collected and published, the following circumstances should be taken into account: The provincial governments of Spain are somewhat peculiar, in that the civil governors change with the changes which take place in the Government at Madrid, so that the political constitution of the provincial governments is always a reflex of that of the central Government at Madrid. Moreover, the political sentiment of the provincial government is also more or less perfectly reflected by the local governments of the towns of the province.

"The hostility of the Minister of the Interior at Madrid to Dr. Ferrán, and his attempts at the prevention of cholera by inoculation, is a well-known fact now generally admitted; and the hostility which Dr. Ferrán met with from the civil governor of the province of Valencia was even greater than that manifested by the Minister of the Interior himself.

"The official statistics of the Ferrán inoculations are in the first place signed by the physicians of the locality; and in the next place by the judge of the municipal court, and sometimes also by the president judge of the judicial district, by the parochial priest, and by the mayor of the municipality, whose signatures and seals are attested by an authorized notary public.

"It must, therefore, be obvious that the charge made by the French Commission, which has been so constantly reiterated everywhere, that the public statistics of the anti-choleraic inoculations are void of official character and are to be regarded as *ex-parte* testimony of the partisans of Ferrán, cannot apply to official statistics which were collected under the supervision of the municipal authorities of the villages wherein the inoculations were performed, and attested not only by the local judicial officers and the parochial priests, but also by the political officers—that is to say, the secretaries and the mayors of the municipalities; for it must be admitted that neither

the political officers of the municipalities nor of the provincial governments, any more than the parochial priests, can reasonably be charged with being the partisans or friends of Ferrán—the Minister of the Interior continuing during the time of collection of these official statistics to be hostile to the claims of Ferrán. It therefore follows that the attack upon the statistics of the inoculations made by the French Commission, and so widely accepted by the medical world as conclusive, does not apply to the official statistics of which we are speaking. And, in view of this fact, the evidence as to the efficiency and harmlessness of the anti-choleraic inoculations should be re-examined. As I have already said, the results of the preventive inoculations of Ferrán, as set forth in the official statistics, appear to very strongly support his claim of the protective value of the inoculations. In view of the great importance of this whole subject I have determined to place these statistics in this report for the benefit of the readers of the English language, in order that they may judge for themselves of the facts as they appear to be recorded.

"From the Government statistics of cholera throughout the province of Valencia, it appears that among the villages invaded there were 62 attacks per thousand of the population, and 31 deaths per thousand, which gives a mortality of fifty per cent. of those attacked. It appears from analysis of the published official statistics of cholera in twenty-two towns where inoculation was performed the inhabitants were divided as follows: 104,561 not inoculated; 30,491 inoculated. Of the latter there were 387 attacks of cholera, or 12 per thousand, and 104 deaths, or 3 per thousand; the mortality of those attacked being twenty-five per cent. Of the former there were 8,406 attacks, or 77 per thousand, and 3,512 deaths, or 33 per thousand, being a mortality of those attacked of forty-three per cent. It appears, therefore, that among the population of villages wherein anti-choleraic inoculations had been more or less extensively performed the liabil-

ity of the inoculated to attacks of cholera was 6.06 times less than that of the non-inoculated, whilst the liability of the inoculated to death by cholera was 9.87 times less than that of the non-inoculated. These figures are based exclusively upon the data furnished by inoculations, the reinoculations being left out of consideration, because they are much less numerous, although from the records of the inoculations it would seem that the liability of attack, and especially of death by cholera is many times less among them than among those inoculated a single time.

"The charge has also been made with respect to the published records of the inoculations that the hygienic and physical condition of the subjects of inoculation have not been sufficiently indicated in the records, and that the vast majority of those profiting by the opportunity to receive the anti-choleraic inoculations were of the middle and upper classes, and therefore not of that class of the inhabitants who are notoriously most liable to attack and death from cholera. This criticism may have some justness as respects some, perhaps many, of the villages where inoculations were performed; but there are certainly many of the villages wherein the results of the inoculation seemed to be most positively in favor of the claim of Ferrán where this criticism cannot hold. I refer to villages wherein three-fourths or four-fifths of the inhabitants were inoculated, leaving only the fraction of the population non-inoculated. Even in the absence of any special notes indicating the social conditions and hygienic surroundings of the inoculated in these villages it is ridiculous to assume that the vast majority of these were people of the middle and upper classes, and were therefore but little liable to attack and death by cholera. Any one acquainted with the character of the Spanish population as it exists in the rural villages, will admit at once that the vast majority of this population consists of the wretched and the poor, who live under the most unhygienic and unsalubrious con-

ditions, and therefore are of that class most liable to suffer from cholera.

"There is still another result of the preventive inoculations of Ferrán apparently shown by these statistics. I refer to the apparent marked shortening of the course of the epidemic after a large percentage of the inhabitants have become inoculated. It would seem, therefore, from analysis of the official statistics, that the practice of the anti-choleraic inoculation after the method of Ferrán, besides giving the subject inoculated a considerable immunity from attack and death by cholera, furnishes a means of bringing an epidemic rapidly to an end."

With reference to Haffkine's method of inoculation we cannot do better than to quote from a lecture which he gave in London, in 1893:

"In the research that I have done at the Pasteur Institute on vaccination against Asiatic cholera I have chosen for my starting-point the inoculation of the animal into the peritoneal cavity. Starting from this point I have worked out a method which permits the culture of the microbe in the animal organism in a state of purity during indefinite generations, the exaltation of it to a well-determined maximum of strength, and keeping it at the same degree of virulence for an unlimited period of time.

"This method is illustrated by three series of experiments which were the subject of our publications in the *Comptes-rendus de la Société de Biologie* of Paris, and which are:

"1. Giving the first animal a dose larger than the fatal dose, and killing this animal in a sufficiently short space of time to be able to find the more resisting microbes.

"2. To expose the exudation taken from the peritoneal cavity to the air for several hours.

"3. Then to transfer this exudation to the next animal, of large or small size, according to the concentration of the exudation.

"In the hands of a number of other experimenters this method has given the same results and showed a perfect consistency.

"The properties of the virus which is obtained in this manner of cultivation are as follows: Upon intraperitoneal inoculation it kills guinea-pigs regularly in the space of about eight hours, and the fatal dose for this animal is reduced to about twenty times less than that which it would have been necessary to take for the microbe with which I started. The same inoculation kills rabbits and pigeons with a dose which would have been perfectly harmless at the beginning of the experiments. It kills guinea-pigs by intramuscular inoculation.

"The subcutaneous inoculation brings about the formation of a large œdema, which tends toward sequestration of a whole part of the cutaneous tissues and to the formation of a wide open wound, which is cured in from two to three weeks.

"The basis of anticholeraic vaccination is founded on the virus obtained in the manner we have just described.

"This virus, injected under the skin of a healthy animal, gives it, after several days, immunity from all choleraic contamination, in whatever manner this may arise; that is to say, if an animal that has been thus treated be taken, and an attempt made to infect it either by the digestive canal, by neutralization of the gastric juice and the injection of opium into the peritoneum, or by the introduction of the microbe into the intestines by the method of Nicati and Rietsch, or by intramuscular inoculation, or finally, by intraperitoneal injection, the most terrible of all, it resists, whilst the control animals succumb.

"Anticholeraic vaccination of animals in this manner is then definitely established. But the operation described cannot be, such as it is, applied to man. The wound following on the subcutaneous inoculation is terrible to look at, and, in all probability, extremely painful. Besides, although it does not in itself present any danger

to the health of the individual, it exposes him to all the complications inseparable from an open wound.

"This power of producing necrosis of the cutaneous tissues has been removed from the exalted vaccine by cultivating it at a temperature of 39° C., and in an atmosphere constantly aërated. Under these conditions the first generations of the cholera microbe would die rapidly, in an interval of two to three days, and therefore care must be taken to sow them again in new media immediately before death, and after a series of generations of this kind a culture is obtained which, if injected under the skin of animals, even in exaggerated doses, only produces a passing œdema, and prepares the organism in such a manner that the injection of exalted virus, the definite vaccine, only produces a local reaction of the slightest description.

"VACCINATION BY FIXED VACCINE.

"The method of vaccination thus worked out comprises, then, two vaccines—a mild vaccine, obtained by weakening the fixed virus; and a strengthened vaccine, which is presented by the virus itself. It is easy to understand why to obtain the weakened vaccine we do not use an ordinary virus, but a virus the nature of which has been previously fixed in the laboratory. It is because the virus, such as is found in the natural state, especially when it has a saprophytic phase of development, presents such pathogenic differences that there is no certainty in its application. Respecting this we need only recall the story of variolization, and the great danger that an individual incurred when the infectious substance from a slightly attacked subject was transferred to him. The mildness or the gravity of an infection does not depend only on the veritable strength of the contagious substance, but upon the resistance of the individual from whom it is taken. Thus it happened that in taking vaccine lymph from a subject lightly affected, a very

weak substance was sometimes produced, which was incapable of producing a protective action; and sometimes a lymph of such strength that it killed less resistant individuals. The great benefit of Jenner's discovery lay in that it precisely indicated a substance fixed by passages through animals, and of a virulence below that which is fatal to the human organism. Another example is given in the method of Toussaint of vaccination against anthrax, the first of its kind, which has been obliged to make way for the method of M. Pasteur, for the sole reason that the latter, based upon virus of a fixed nature, presented an absolute certainty in its results which was wanting in the other. Finally, in the history of cholera itself I may recall the attempt made in 1885 by Dr. Ferrán, of Barcelona, who, with the object of preserving the population of the Peninsula from the epidemic of cholera, made injections in his patients of the ordinary virus taken from dead bodies and cultivated in the laboratory. The statistics of the results obtained by this means showed such uncertainty that no one dared to recommend this operation to his country in spite of the very numerous trials made in Spain.

"The possibility of treating the animal organism by vaccines of an absolutely fixed nature, prepared by means of special operations, constitutes, on the contrary, the basis of the Pasteurian method, and here lies the whole secret and the sole guarantee of the success of its application.

"APPLICATION OF THE METHOD TO MAN.

"The method of anticholeraic vaccination, worked out by experiments on guinea-pigs, was tried upon rabbits and pigeons before it was applied to man. These animals were chosen in order to have subjects very differently organized, and in order to be able to generalize the conclusions, and to be able to extend them to the human organism.

"The result obtained on all these animals being abso-

lutely the same, it was decided to apply the operation to man.

"The symptoms produced by this operation have been described in several scientific magazines. The method has been tried at Paris, at Cherbourg, and at Moscow, on about fifty persons of both sexes, between the ages of nineteen and sixty-eight, of French, Swiss, Russian, English, and American nationality.

"In every case the method has shown itself absolutely harmless to health, and the symptoms that it evoked were a rise of temperature, a local sensitiveness at the place of inoculation, and the formation of a transitory œdema at the same place. The first sensations are felt about two or three hours after inoculation; fever and general indisposition disappear after twenty-four to thirty-six hours; the sensitiveness and œdema last, gradually dying away in from three to four days. The symptoms following the second inoculation were generally rather more marked, but of shorter duration. The whole recalls the sensation of a bad cold in the head, lasting about one or two days.

"The microbes introduced under the skin do not propagate, but after a certain time they die and disappear. It is the substances which they contain, and which are set free when they die, that act upon the animal organism and confer immunity upon it. It is found that the same result can be obtained if the microbe be killed before inoculation, and if their dead bodies only be injected. Thus I have been able to prepare vaccines preserved in weak solutions of carbolic acid. In this the microbes die at the end of several hours, and the vaccine so prepared has been found still efficacious six months after its preparation. It is evident that there is much advantage in this state of preservation of the microbes. They can be used by persons having no bacteriological training, and the absence of every living organism makes them perfectly safe. The carbolic acid that they contain preserves them against any invasion of other microbes.

Finally, as they can be kept for several months, their preparation can be intrusted to a central laboratory, whence the vaccine *ampoules* can be sent out to operators. But it may be presumed that immunity given by these preserved vaccines will not equal in persistency that produced by living ones, and as the method is not yet backed up by established statistics, it is better that vaccinations should be done as much as possible with living virus, so as to obtain the most conclusive results.

"As to the length of time that immunity produced by living vaccine lasts, we have not yet at the laboratory animals that have been inoculated at a very distant date; those upon which we experimented dated from, at most, four months and a half. At the end of this time their immunity was found to be still perfect, and we do not despair of its lasting much longer yet.

"HARMLESSNESS OF THE METHOD.

"The inoculations upon man, added to the hundreds of experiments that we have made upon animals, testify to the perfect harmlessness of these operations, and there is no difficulty in proving their efficacy by experiment, no matter on what species of animal. We have taken twelve guinea-pigs, and vaccinated six of them with vaccines preserved in carbolic acid since September 8th last. Yesterday, at five o'clock, six days after the first vaccination, we injected into the peritoneal cavity of all the non-vaccinated animals a fatal dose of virus, and into the vaccinated animals we injected a double dose. The six vaccinated animals are perfectly well, while of the others two have already died of choleraic poisoning, two are very ill, and the others will certainly soon become so. But it is evident that I cannot perform a like experiment on man (but, however, this would be the only means of being able to give a definite experimental demonstration)."

Further details as to the method are given by Woodhead in the "Edinburgh Hospital Reports," as follows:

"In order to be absolutely certain that the virus is pure, M. Haffkine makes cultivations before each inoculation of the human subject, by Roux and Yersin's method, one devised for the separation of the diphtheria bacillus. A small drop of the *virus exalté* is taken on a spatula-shaped needle, and streak after streak is made with the flat of this needle on the surface of the agar in the tubes, a couple of tubes being used, so that twelve streaks perhaps, in all, are made without the needle being recharged; in the earlier streaks, of course, the seed bacilli are so close together that a continuous line of colonies makes its appearance; but along the course of the later streaks, colonies, with distinct intervals between them, are developed; part of one of these is examined under the microscope, in order to determine that it is made up only of comma bacilli, and then the other part is used for seed material for a tube-culture preparatory to inoculation.

"The inoculation itself is an exceedingly simple process; the needle and the syringe are boiled; the tube containing the material to be used for inoculation receives a syringeful or pipetteful of sterilized beef-broth, then with a platinum needle the culture is thoroughly mixed with this broth, so that a kind of emulsion is prepared; this emulsion is drawn up in a sterilized pipette, and is then passed into a sterilized conical glass covered with sterilized paper. If a sixth of the culture is to be introduced, two more syringefuls or pipettefuls of broth are to be added, so that we now have three in all; if an eighth, three are added, and so on; the whole is mixed, and then half a syringeful is taken for use for each patient. In inoculating, the skin, just above the crest of the ilium, is thoroughly cleansed with five per cent. solution of carbolic acid, the attenuated virus is inoculated on the left side, and then after an interval of four or five days the second vaccine, or the more virulent form, is inoculated

on the right side. After inoculation everything that has been used is thoroughly boiled, the skin of the patient is again washed with five per cent. carbolic acid, and the table is washed down with the same solution."

Haffkine commenced his experiments on man by inoculating himself, and has repeated the inoculation three times. He next inoculated about fifty individuals in Paris, Cherbourg, and Moscow, and demonstrated in a satisfactory way that the inoculations are without danger.

A first inoculation in an unprotected person is said to give rise to some malaise and febrile reaction, to pain and tumefaction at the point of inoculation, and swelling of the neighboring glands. The second inoculation with a strong virus, made after an interval of six days, causes also some elevation of temperature, but no swelling at the point of inoculation. This slight reaction from a strong virus is supposed to be satisfactory evidence of a certain degree of immunity as a result of the first inoculation.

The results of the protective inoculations by Haffkine's method, which have been practised in India, indicate that these inoculations have a real value, but that immunity is not immediately established, and consequently that during an epidemic a certain number of fatal cases may be expected among the inoculated as well as among the non-inoculated. This is illustrated by the results of inoculations made among the prisoners in Gaya jail (1894), reported by Surgeon-Major Macrae, I.M.S., from whose report we quote as follows:

"Cholera broke out in the Gaya jail on the 9th of July, and from that date to the 2d August 34 cases occurred, with 20 deaths, there being on date of first attack 422 prisoners in jail. The disease was clearly traceable

to importation, but its diffusion among the prisoners was a question of much greater difficulty. The sanitary condition of the jail is excellent; it was built quite recently, on the latest plans, and is generally considered a model jail. The water-supply, which is from a well, is of excellent quality and protected from pollution, and it is believed that the spread of the disease was largely due to the agency of flies finding access to food and milk after being in contact with cholera poison, and contaminating them. From the 9th to the 17th July, 6 cases occurred, with 5 deaths.

"Many of the prisoners on being told about preventive inoculation wished to be inoculated, and M. Haffkine, who had previously been communicated with, and whose zeal and enthusiasm in the cause that he so well advocates are beyond praise, arrived here on the 18th July, and in the presence of Surgeon-Colonel Harvey, who kindly assisted, and myself, inoculated 147 prisoners, and on the 19th 68, making a total of 215 out of 433 present in the jail on that date.

"Being purely voluntary, no selection of prisoners was possible; but all classes in the jail were represented, male and female, old and young, habituals and less frequent offenders, strong and weakly, convalescent and even hospital patients sent their representatives. No difference of any kind was made between inoculated and non-inoculated prisoners; they were under absolutely identical conditions as regards food, water, accommodation, etc., in short, in every possible respect.

"As, owing to the progress of the epidemic, a large number of prisoners were removed from the jail into camp, it will be found convenient to consider the effect produced by the anticholera inoculation under three headings:

"(*a*) *The first* will include the period from the 18th July, the date of first inoculations, to the 24th July, the date on which final reinoculations were made, and refers to all the prisoners.

"(*b*) *The second* concerns the prisoners who remained in jail after the majority were removed into camp, and comprises the period from the 25th July, to the 2d August, on which date the final case occurred among this body of prisoners.

"(*c*) *The third* refers to the body of prisoners who were moved into camp on the 25th July, and includes the period between that date and the 1st August, when the final case occurred among this body.

	Average present.	Cholera.	Percentage of average strength.	Deaths.	Percentage of average strength.	Percentage of deaths to cases.
			No. I.			
Inoculated.....	211.2	5	2.37	4	1.89	80.0
Not inoculated.....	209.0	7	3.34	5	2.39	71.42
			No. II.			
Inoculated.....	32.5	1	3.07	Nil.	Nil.	Nil.
Not inoculated.....	48.55	7	14.42	3	6.18	42.86
			No. III.			
Inoculated.....	171.42	2	1.16	1	0.58	50.0
Not inoculated.....	146.5	6	4.09	2	1.36	33.33

"The conclusions to be drawn from the results above recorded appear to me to be that for the first few days the inoculations have scarcely any protective influence; then their effect seems to gradually increase. M. Haffkine in his publications has laid stress on the fact that he anticipates a period of ten days would elapse from date of first inoculations before the full effect would be obtained.

	During the first five days after first inoculation.		First three days after second inoculation.		Last six days.	
	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
Inoculated.....	5	4	3	1	Nil.	Nil.
Not inoculated.....	7	5	5	3	8	2

"Further observations are necessary to prove whether the inoculations as now practised will prove of lasting benefit; the results obtained in Gaya jail seem to me to justify the conclusion that their temporary beneficial effect is undoubted.

"I have been informed by M. Haffkine that he proposes to introduce a certain modification of his method, with the object of affording protection to patients during the ten days necessary for the action of his vaccines. I think there is every reason to believe that better results would have been obtained here had the inoculations been performed at an earlier period instead of during the epidemic."

In a recent paper (*British Medical Journal*, January 26, 1895) Haffkine gives the following summary of his inoculations in India:

"*Table showing the total number of persons on whom observations have been made in Calcutta, Gaya, Cawnpore, and Lucknow.*

	Number.	Cases.	Percentage of Cases to Strength.	Deaths.	Percentage of Deaths to Strength.
Non-inoculated.	1,735	174	10.63	113	6.51
Inoculated.....	500	21	4.20	19	3.80
Total.....	2,235	195		132	

Other methods of producing immunity in man have been proposed, and recent experiments indicate that this may be accomplished through the digestive tract by the ingestion of considerable quantities of sterilized cultures. Thus Klemperer (1892) has obtained results which seem to show that immunity in man may be induced not only by the subcutaneous injection of virulent cultures but also by the subcutaneous injection of the milk of immu-

nized goats and by the ingestion of cultures sterilized by heat. The degree of immunity, as determined by the activity of the blood-serum of the immune individual for the protection of guinea-pigs, is considerably less, however, than when repeated injections of virulent cultures have been made. The blood-serum of individuals made immune by the last-mentioned method is said by Klemperer to protect guinea-pigs when injected into the cavity of the abdomen in the dose of 0.005 c.c. And the injection of 5 c.c. of milk from an immunized goat is said to confer such an immunity that 0.25 c.c. of blood-serum from the immune individual is sufficient to protect a guinea-pig from cholera cultures. In this connection attention is called to the results obtained in more recent experiments by Pfeiffer (p. 118).

Sawtschenko and Sabolotny (1893), as a result of a series of experiments made upon themselves and laboratory assistants, arrive at the following conclusions :

"1. After the ingestion of sterilized (by heat) and subsequently carbolized agar cultures of cholera bacteria the serum of man acquires an immunizing property as regards the cholera vibrio.

"2. As a result of the ingestion of sterilized agar cultures the individual is protected from infection with virulent cultures of the cholera vibrio by way of the intestine.

"3. The discharges of individuals immune against cholera, and to all outward appearance in perfect health, may contain a great number of cholera vibrios (in case they are in any way introduced into the intestine) and may thus serve to propagate the malady."

Serum-therapy.

The results of experiments already referred to are favorable to the view that the serum of immune individuals

(man or lower animals) contains a substance (antitoxin ?) which would probably be useful in the treatment of cholera if a practical method of obtaining it in sufficient quantity should be devised. This view receives support from the experiments of Pawlowsky and Buchstab (1893) and of Fedoroff (1893). The first-named bacteriologists, after demonstrating the fact that guinea-pigs, rabbits, and dogs could readily be immunized by the methods heretofore referred to, proceeded to make further experiments with the serum of immunized dogs. The serum from these animals proved to have a decided bactericidal action for cholera cultures, and when injected into the peritoneal cavity of rabbits or guinea-pigs, in doses of 5 c.c., it made these animals immune against the usually fatal doses of virulent cultures of the cholera spirillum. The dogs were immunized by first injecting attenuated cultures subcutaneously in doses of 10 c.c., and in following this with more virulent cultures and larger doses, until at last the animal received 90 c.c. of a virulent culture. The immunizing value of the serum obtained from a dog treated in this way was estimated at 1 to 130,000. Five c.c. of a virulent culture mixed with 1 c.c. of this serum produced no effect when injected into the peritoneal cavity of guinea-pigs. And the curative value of the serum, when injected subcutaneously two to five hours after intraperitoneal infection with 5 c.c. of a virulent culture, was shown by the fact that out of 16 animals treated in this way 12 survived, while the control animals all died. The serum was injected by the authors referred to beneath their own skin in doses of 1 c.c., and proved to have no noticeable effect upon the pulse or temperature.

Fedoroff (1893) did not succeed in obtaining a serum of as high an immunizing value as that obtained by the au-

thors last referred to, or by Klemperer, but he arrives at the conclusion that "it is a settled fact that animals may be rendered immune against cholera in a surprisingly short time by means of the blood-serum of other immune animals. Also that blood-serum therapy in Asiatic cholera seems much more promising than the method proposed by Brieger, Kitasato, and Wassermann—injections of cultures in thymus bouillon."

Dr. Freymuth, of Danzig, in the *Deutsche Medicinische Wochenschrift* of October 25, 1894, reports three cases of cholera treated by subcutaneous injections of blood-serum from individuals who had recently suffered attacks.

The first case, a female, was in a complete state of collapse on August 13th, when 10 c.c. of serum was injected under the skin between the shoulders. No improvement was noted as a result of the injection. The following day a dose of 30 c.c. was given in the same way. No improvement. The following day a dose of 50 c.c. was followed by apparent improvement, but this was not maintained, and the patient died on the 18th.

In the other two cases recovery occurred. One received in all 80 c.c. of serum, and the other 20. As the reporter observes, the number of cases is too small to justify any conclusions as to the value of the treatment; but they are of interest as being the first cases reported in which this mode of treatment was resorted to, and as showing, at least, that it is harmless and not difficult to carry out when there are persons at hand who have recovered from a recent attack, and are willing to spare the blood.

With reference to the practical value of protective inoculations for the prevention of cholera epidemics, the writer sees no reason to modify the views expressed in

his paper on "Protective Inoculations in Infectious Diseases," read at the twentieth annual meeting of the American Public Health Association (1892). Speaking of Ferrán's inoculations, and the more recent experiments of Haffkine, I say:

"Whether this method will be found to have any great practical value, can only be determined by more extended experiments. But in view of the fact that other measures of prophylaxis, well known to sanitarians, are sufficient for the prevention of cholera epidemics, and that nurses and others who necessarily come in contact with cholera patients are not likely to contract the disease if they use proper precautions with reference to their food and drink, the disinfection of their hands, etc., we doubt whether protective inoculations will ever come into general use as a measure of prophylaxis against this disease. Certainly they cannot take the place of those sanitary measures which have been proved to be sufficient for the prevention of epidemics, namely, exclusion by a proper inspection service at ports of entry ('quarantine'), isolation of the sick, disinfection of excreta, general sanitary police of exposed towns and cities, boiling the water used for drinking purposes, etc. Still, under certain circumstances, protective inoculations may have considerable practical importance, and the experiments now being made have evidently great scientific interest in connection with the question of acquired immunity."

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IV.

DIPHTHERIA.

Diphtheria is generally recognized by physicians as a specific infectious disease, and, owing to its wide prevalence and fatal character, a precise knowledge of its etiology is of the greatest importance. Until, as a result of recent researches, this was determined, pathologists were in doubt as to whether diphtheria should be considered as primarily a local infection, or whether the local manifestations were secondary to a general systemic infection. But this question appears now to be definitely settled in favor of the former view. We have to-day a very precise knowledge of the specific infecting agent, and have evidence that it produces during its growth a very potent toxic substance, the absorption of which from the seat of local infection accounts in a satisfactory manner for the general symptoms of the disease, which are due to toxæmia and not to an invasion of the blood and tissues by the pathogenic microörganism producing it.

Numerous researches by competent bacteriologists have failed to demonstrate the presence of bacteria in the blood of patients suffering from diphtheria, but various microörganisms have been obtained in cultures from diphtheritic pseudo-membranes, and may be demonstrated by the microscopical examination of stained preparations. Among these are the well-known pus organ-

isms, and especially the streptococcus pyogenes, which appears to be very commonly present, and is the active agent in the production of certain forms of pseudo-diphtheria. But the malignant, specific diphtheria, so well known in this country and in Europe, has been demonstrated by the recent researches of bacteriologists to be due to a bacillus, first recognized by Klebs in stained preparations of diphtheritic false membranes (1883), and cultivated and described by Löffler in 1884. In his first publication Löffler did not claim to have fully demonstrated the etiological relation of this bacillus, but this appears to be fully established by subsequent researches.

In his first research Löffler studied twenty-five cases, and in the greater number of them found in stained preparations the bacillus previously described by Klebs. From six of these cases he obtained it in pure cultures, and by experiments on pigeons, chickens, rabbits, and guinea-pigs proved that it gave rise to a diphtheritic inflammation when inoculated into the mucous membrane of the trachea, conjunctiva, pharynx, or vagina. In a second communication Löffler reported his success in finding the same bacillus in ten additional cases, and also that he had isolated from the same source a non-pathogenic bacillus which resembled it very closely. This pseudo-diphtheria bacillus has since been found by other bacteriologists (Von Hoffman, Roux and Yersin), and it is uncertain whether it is to be considered a distinct species, or a non-pathogenic variety of the diphtheria bacillus as maintained by Roux and Yersin. But its occasional presence does not invalidate the very positive experimental evidence relating to the specific pathogenic power of the true diphtheria bacillus.

According to Roux and Yersin, "attenuated varieties"

of the diphtheria bacillus may be obtained by cultivating it at a temperature of 39.5° to 40° C. in a current of air; and these authors suggest that a similar attenuation of pathogenic power may occur in the fauces of convalescents from the disease, and that possibly the similar non-pathogenic bacilli which have been described by various investigators have originated in this way from the true diphtheria bacillus. These authors further state, in favor of this view, that from diphtheritic false membrane, preserved by them in a desiccated condition for five months, they obtained numerous colonies of the bacillus in question, but that the cultures were destitute of pathogenic virulence. They say:

"It is then possible, by commencing with a virulent bacillus of diphtheria, to obtain artificially a bacillus without virulence, quite similar to the attenuated bacilli which may be obtained from a benign diphtheritic angina, or even from the mouth of certain persons in good health. This microbe, obtained artificially, resembles completely the pseudo-diphtheritic bacillus; like it, it grows more abundantly at a low temperature; it renders bouillon more rapidly alkaline; it grows with difficulty in the absence of oxygen."

Subcutaneous inoculations in guinea-pigs of a small quantity of a pure culture of the bacillus (0.1 to 0.5 c.c. of a bouillon culture) cause death in from one to four or five days. The usual changes observed at the autopsy are—

"An extensive local œdema, with more or less hyperæmia and ecchymosis at the site of inoculation, frequently swollen and reddened lymphatic glands, increased serous fluid in the peritoneum, pleura, and pericardium, enlarged and hemorrhagic suprarenal cap-

sules, occasionally slightly swollen spleen, sometimes fatty degenerations in the liver, kidney, and myocardium. We have always found the Löffler bacilli at the seat of inoculation most abundant in a grayish-white, fibrino-purulent exudate present at the point of inoculation, and becoming fewer at a distance from this, so that the more remote parts of the œdematous fluid do not contain any bacilli" (Welch and Abbott).

The authors quoted agree with Löffler and others in stating that the bacillus is only found at the point of inoculation. In all cases their cultures from the blood and from the various organs gave a negative result.

Rabbits are not so susceptible, and may recover after the subcutaneous inoculation of very small doses, but usually die in from four to twenty days when 2 to 4 c.c. of a bouillon culture have been introduced beneath the skin. In these animals, also, there is an extensive local œdema, enlargement of the neighboring lymphatic glands, and a fatty degeneration of the liver. Roux and Yersin have shown that in rabbits, when death does not ensue too quickly, paralysis of the posterior extremities frequently occurs, thus completing the experimental proof of the specific pathogenic power of pure cultures of this bacillus.

Similar symptoms are produced in pigeons by the subcutaneous inoculation of 0.5 c.c. or more, but they commonly recover when the quantity is reduced to 0.2 c.c. (Roux and Yersin).

The rat and the mouse have a remarkable immunity from the effects of this poison. Thus, according to Roux and Yersin, a dose of 2 c.c., which would kill in sixty hours a rabbit weighing 3 kilogr., is without effect upon a mouse which weighs only 10 gm.

Old cultures are somewhat less virulent than fresh ones, but when replanted in a fresh culture medium they manifest their original virulence. Thus a culture upon blood-serum which was five months old was found by Roux and Yersin to kill a guinea-pig in five days, but when replanted it killed a second animal of the same species in twenty-four hours.

Evidently a microörganism which destroys the life of a susceptible animal when injected beneath its skin in small quantity, and which nevertheless is only found in the vicinity of the point of inoculation, must owe its pathogenic power to the formation of some potent toxic substance, which, being absorbed, gives rise to toxæmia and death. This inference in the case of the diphtheria bacillus is fully sustained by the results of recent experimental investigations. Roux and Yersin (1888) first demonstrated the pathogenic power of cultures which had been filtered through porous porcelain. Old cultures were found by these experimenters to contain more of the toxic substance than recent ones, and to cause the death of a guinea-pig in a dose of 2 c.c. in less than twenty-four hours. The filtered cultures produced in these animals the same effects as those containing the bacilli—local œdema, hemorrhagic congestion of the organs, effusion into the pleural cavity. Somewhat larger doses were fatal to rabbits, and a few drops injected subcutaneously sufficed to kill a small bird within a few hours. In their second paper (1889) the authors mentioned state that so long as the reaction of a culture in bouillon is acid, its toxic power is comparatively slight, but that in old cultures the reaction is alkaline, and in these the toxic potency is greatly augmented. With such a culture, filtered after having been kept for thirty days,

a dose of one-eighth of a cubic centimetre injected subcutaneously, sufficed to kill a guinea-pig; and in larger amounts it proved to be fatal to dogs when injected directly into the circulation through a vein.

The same authors, in discussing the nature of the poison in their filtered cultures, infer that it is related to the diastases, and state that its toxic potency is very much reduced by exposure to a comparatively low temperature—58° C., for two hours—and completely destroyed by the boiling temperature—100° C., for twenty minutes. It was found to be insoluble in alcohol, and the precipitate obtained by adding alcohol to an old culture proved to contain the toxic substance. Löffler also has obtained, by adding five volumes of alcohol to one of a pure culture, a white precipitate, soluble in water, which killed rabbits in the dose of 0.1 to 0.2 gm. when injected beneath the skin of these animals. It gave rise to a local œdema and necrosis of the skin in the vicinity of the point of inoculation, and to hyperæmia of the internal organs. This deadly *toxin* appears to be an albuminoid substance, but its exact chemical composition has not yet been determined.

Brieger and Fränkel (1891) obtained results corresponding with those previously reported by Roux and Yersin. Their researches showed that the toxic substance contained in diphtheria cultures is destroyed by a temperature of 60° C.; that it is soluble in water, and insoluble in alcohol; that it does not pass through a dialyzing membrane, and has not the chemical characters of the ptomaïnes or toxins, but is an albuminous body—a toxalbumin. It was obtained by the authors named by precipitation with slightly acidified (acetic acid) alcohol; the precipitate, after being washed in a dialyzer and dried

in a vacuum at a temperature of 40° C., was a snow-white, amorphous, crumbling mass.

Wassermann and Proskauer (1892) found that the alcoholic precipitate from diphtheria cultures contains two different substances, which are distinguished by their different degrees of solubility in diluted and absolute alcohol; both, however, give the usual reactions of albuminous bodies, and pass very slowly through a dialyzing membrane. Only one of these substances possesses toxic properties. After the removal of peptone and globulin from the filtered cultures, these were evaporated and a precipitate obtained of one of the albuminous substances by means of sixty per cent. to seventy per cent. alcohol. The other substance remained in solution, and was subsequently obtained by precipitation with absolute alcohol. The substance first obtained by this method is toxic, and the other precipitate is not. The authors named succeeded in killing rabbits with the toxalbumin obtained in this way, but were not able to produce immunity in these animals by the injection of non-fatal doses. Fränkel (1891) had previously reported his failure to immunize guinea-pigs by the injection of the dry precipitate, obtained in his experiments from diphtheria cultures; but when filtered cultures, or cultures sterilized by heat (55° C. for one hour), were injected into these animals, they showed an increased resistance to the pathogenic action of virulent cultures. Still better results were obtained when 10 c.c. of a bouillon culture, heated to 100° C., were injected subcutaneously, but still this method was not entirely reliable. But true immunity was established by injecting into the peritoneal cavity 10 to 20 c.c. of a bouillon culture heated to 65° to 70° C. for one hour. The immunity was not fully established until

about fourteen days after the protective inoculation. Fränkel arrives at the conclusion that the cultures must contain an immunizing substance as well as a toxic proteid, as the diphtheria toxalbumin is destroyed by the temperature (65° to 70° C.) used in the preparation of his cultures for producing immunity.

Behring, in the same year (1891), commenced his experiments upon diphtheria immunity. Guinea-pigs were made immune by the use of sterilized cultures, and by inoculations with virulent cultures, four weeks old, to which iodine terchloride had been added in the proportion of 1 to 500—the mixture was allowed to stand for sixteen hours. Animals were also immunized by injecting beneath the skin a virulent culture of the bacillus, and then treating them with subcutaneous injections of iodine terchloride (2 c.c.), which was thrown under the skin for three days in succession in the vicinity of the point of inoculation. The guinea-pigs treated in this way remained sick for some time, but finally recovered and were subsequently immune. Still better results were obtained when rabbits were subjected to the same treatment. The animals were immune against the toxic action of sterilized cultures, as well as against infection by virulent diphtheria bacilli.

In subsequent experiments (1892) Behring and Wernicke used cultures which had been attenuated by contact with iodine terchloride for from thirty-six to forty-eight hours, and proved that the method could be successfully employed in immunizing sheep; and the fact was ascertained that blood-serum from an immune animal could be used with success in arresting diphtheritic infection in susceptible animals. To preserve the serum, which they obtained from immunized sheep, rabbits, and

guinea-pigs, they added to it 0.5 per cent. of pure carbolic acid. For producing immunity they found that a smaller amount of serum was required than was necessary for the cure of an animal already infected. If the injection was made immediately after infection from one and a half to two times the amount was required; eight hours after infection the amount was three times as great, and twenty-four to thirty-six hours after infection the dose required was eight times the immunizing dose.

The immunizing value of blood-serum from different animals was estimated by finding the smallest dose which would protect an animal from fatal infection by the minimum lethal dose of a culture, the toxic potency of which had been carefully determined. The value is expressed in figures which give the proportion required compared with the body-weight of the animal. Thus an immunizing value of 100 would mean that 1 gm. of the serum is sufficient to protect an animal weighing 100 gm. from the fatal effect which would be produced in a control animal of the same weight by infection with a virulent culture of the diphtheria bacillus in the minimum dose required to produce this result. The cultures employed are made in bouillon containing one per cent. of peptone; they are inoculated from agar cultures and are kept in the incubating oven for two days. Cultures prepared in this way were found to be quite uniform in their pathogenic virulence as tested upon guinea-pigs. But when cultures are kept for some time there is an increase in virulence. Thus a culture obtained from a fatal case of diphtheria which in 1890 killed guinea-pigs in three to four days, when injected subcutaneously in the dose of 0.1 c.c. (two days old bouillon culture), at the end of a year was fatal to these animals in the dose of 0.025 c.c. This increase

in virulence is ascribed to the fact that the cultures were renewed at long intervals.

More recently (1894) Behring has fixed a standard for what he calls a normal therapeutic serum. This is a serum which when injected into guinea-pigs in the proportion of 1 to 5,000 of body-weight saves the animal from the fatal effects of ten times the minimum dose of a culture in bouillon, two days old, which would kill a control animal not treated.

In a recent communication (November, 1894) Behring states his conclusion that for producing immunity in man, 150 normal antitoxin units should be given, instead of 60 as he had previously recommended.

The serum manufactured under his direction is said (September, 1894) to be of two kinds—one, obtained from the horse, has a value of 60 normal antitoxin units; the other has a value of 140 units. Of the weaker serum Behring says experience has demonstrated that for children under ten years of age 10 c.c. is sufficient to arrest the progress of the disease and effect a cure if given within two or three days from the outset of the attack. For producing immunity in children subject to infection, one-tenth of this amount (1 c.c.) is said to be sufficient. Of the stronger serum 1 c.c. is sufficient to arrest the disease during the incubation period; and, according to Behring, out of 100 cases treated during the first forty-eight hours with the single therapeutic dose (10 c.c. of serum having a value of 60 = 600 normal units) not 5 will die. The later the treatment is commenced the larger will be the dose required. Behring further states that the diphtheria antitoxin has no injurious effect upon animals in the largest doses that have been employed, and that aside from its antitoxic power its properties

are entirely negative so far as living animals are concerned.

Aronson (1893), in experiments on dogs, succeeded in producing immunity by the use of attenuated cultures, or of cultures to which formaldehyd had been added; also by feeding the animal large quantities of diphtheria bouillon; and, finally, by injection of the blood of naturally immune animals (white rats) into which large quantities (10 c.c.) of a virulent culture had been injected. Two months after receiving several such injections it was found that 0.2 gm. of blood-serum from the rat sufficed to save a guinea-pig from fatal infection. In experiments on dogs an immunity was established in six weeks by the injection of a large amount of a virulent culture, which had a value of 1 to 30,000, *i.e.*, 0.01 c.c. of this serum sufficed to protect a guinea-pig weighing 300 gm. From 100 gm. of this serum Aronson claims to have obtained 0.8 gm. of a substance which had a value of 1 to 500,000, as tested in the treatment of an animal which had received ten times the minimum fatal dose of a two days' bouillon culture. A ten per cent. solution of this substance had, therefore, ten times the value of Behring's "normal-serum." The precipitated antitoxin was soluble in water, and more readily in a slightly alkaline solution, and gave all the reactions of an albuminous body. When dried in vacuo at 40° C., and then heated to 102° C., it still retained its antitoxic potency.

Ehrlich, Kossel, and Wassermann (1894) have made experiments upon goats, which they found very susceptible to the action of the diphtheria poison. Sterilized cultures were first injected in gradually increasing amounts and later virulent cultures. In this way they obtained a serum which has a value sixty times that of

Behring's "normal serum." In a subsequent communication (1894) Wassermann gives an account of his experiments with the milk of immunized goats, which contains the antitoxin in considerable quantity, and from which it was obtained in a concentrated form by the following method: The milk is obtained in sterilized vessels and 20 c.c. of normal hydrochloric acid is added to each litre; a sufficient quantity of rennet is then added to coagulate the casein, and this is separated from the liquid, which is then shaken up with chloroform for some time. The liquid is now allowed to stand in order that the butter, which has been dissolved by the chloroform, may sink to the bottom. The clear liquid is then decanted and the antitoxin precipitated from it by means of ammonium sulphate (thirty to thirty-three per cent.). The precipitate is rapidly dried upon porous porcelain plates, in vacuo, and then dissolved in water in the proportion of 10 parts for 100 of milk first employed—a concentration to one-tenth. Of this solution 0.125 c.c. was found to neutralize 0.9 c.c. of a toxin which killed guinea-pigs weighing 500 gm. in the dose of 0.1 c.c. This toxin was an old bouillon culture of the diphtheria bacillus to which 0.5 per cent. of carbolic acid had been added to preserve it. In a communication of the same date Ehrlich and Wassermann report that they have for some time had a cow immunized to such a degree that 1 c.c. of its milk protects guinea-pigs from the fatal effects of 0.9 c.c. of the above-mentioned toxin. The antitoxic value of the milk of an immunized cow or goat, as compared with that of its blood, is estimated by Ehrlich and Wassermann as from 1 to 15 to 1 to 30—usually about 1 to 20.

Aronson, in testing his antitoxin, uses a bouillon culture of the diphtheria bacillus two and one-half months

old, which he preserves by the addition of 0.3 per cent. of trikresol. He finds that the immunity which results from injections of the antitoxin is established at once; that it is not accompanied by any reaction or symptom of sickness; and that it is of comparatively short duration.

As a result of extended experiments made at the Pasteur Institute in Paris, Roux has perfected the following method for the production of an antitoxin suitable for use in the treatment of diphtheria in man. The horse has been found the most suitable animal for this purpose, on account of his slight susceptibility and the ease with which a high degree of immunity can be established; and because of the large amount of blood that may be drawn without injury to the animal. Roux prepares his toxin by cultivating the diphtheria bacillus in a slightly alkaline bouillon made from beef and containing two per cent. of peptone and 0.5 per cent. of sodium chloride. This medium is placed in flat-bottomed flasks, and should not be more than half an inch in depth. Two glass-tubes pass into the flask, which serve for inlet and outlet tubes to be used in passing a current of air over the cultures. This is commenced when the growth is fairly started, at the end of twenty-four hours, and the air should be moist to prevent the evaporation of the culture. In Roux's laboratory a flask is used which has a tube attached to one side, about an inch from the bottom, and which is known as a Fernbach flask. A flocculent deposit falls to the bottom and gradually accumulates for about a month. This consists of bacilli which have for the most part lost their vitality and are undergoing degeneration. At the end of thirty days, during which time they are kept in an incubating oven at a tempera-

ture of 37° C., the cultures are passed through a Pasteur-Chamberland filter, and 0.5 per cent. of carbolic acid may be added in order to preserve them. This filtrate is so toxic that a dose of 0.1 c.c. will kill a guinea-pig weighing 500 gm. in less than forty-eight hours. A healthy horse is selected and receives at first a dose of 0.5 c.c. of the filtered culture (or of the clear fluid obtained from a culture by decantation and containing 0.5 per cent. of carbolic acid). The dose is gradually increased at intervals of a few days, and is followed each time by some febrile reaction and tumefaction at the point of inoculation. When the reaction is excessive, a little Gram's solution is added to the following dose. The usual plan of treatment is stated by Kinyoun as follows:

"First day, 1 to 2 c.c. of pure toxins, of which 1 to 10 c.c. fatal to a 500-gm. guinea-pig; eighth day, 1 c.c.; fourteenth day, 1½ c.c.; twentieth day, 2 c.c.; twenty-eighth day, 3 c.c.; thirty-third day, 5 c.c.; thirty-eighth day, 8 c.c.; forty-third day, 10 c.c.; forty-seventh day, 20 c.c.; fifty-first day, 30 c.c.; fifty-sixth day, 50 c.c.; sixty-second day, 50 c.c.; sixty-eighth day, 60 c.c.; seventy-fourth day, 100 c.c.; eightieth day, 250 c.c.; eighty-eighth day, 250 c.c.

"When the first injections are given there is quite a marked local and general reaction to the poison; there is an œdema at the point of the injection, which is followed by a distinct inflammatory process—hard in the centre and soft and œdematous at its periphery. The general reaction is manifested by a rise in the temperature, 1° to 2° C., loss of appetite, and occasionally cramps. The reaction must be taken as the guide in the future dosage, and a sufficient time must be allowed to elapse between the injections for the complete recovery from the general and local effects. As the quantity of the toxins is increased the general effects generally decrease, perhaps a

rise of a degree for twenty-four hours. The local effect partakes more of an œdema, and has the character of an inflammation.

"At a certain stage, usually after two months' treatment, when 50 to 60 c.c. can be injected without harm, there is no general reaction, but a large œdema at the site of the injection, which disappears within from twenty-four to forty-eight hours. Toward the last, even when 200 to 300 c.c. are given, there is only an enormous œdema, which disappears within from twelve to eighteen hours. When these inordinately large quantities can be given with only a local reaction being manifest, the horse has come well under the influence, and the blood will be found to be rich in the antitoxin.

"There is a curious fact well worth noting: At the end of the second month of the treatment, when the horse can bear as much as 50 to 60 c.c. of the toxins without discomfort, the blood will be found to contain but little of the antitoxin. The antitoxin only appears after repeated stimulation of the cells (?) by the large and frequent doses of the toxins."

The subcutaneous injections do not yield a serum as rich in the antitoxins as when the toxins are injected directly into the blood-current. When it is desired to do this, toward the last of the treatment the toxins are injected directly into the jugular vein. The process is tedious and requires a longer time, and for practical purposes has not been found so satisfactory as the simple subcutaneous injection. The strength of the serum is tested by using young guinea-pigs of 500 gms. weight. One gr. of the serum usually will protect 50,000 gms. of guinea-pig against a fresh virulent culture of the *Bacillus diphtheriæ*. This is the strength that is used in the hospitals. By the intravenous injections a serum of the protective strength of 1 to 100,000 can be obtained.

When fully immunized from six to eight litres of blood may be taken from a horse at one time, but as a rule it is better not to take more than three. The blood is drawn from the jugular vein, by means of a small trocar and cannula, into wide-mouthed bottles having a capacity of $2\frac{1}{2}$ litres; these are placed in an ice-chest for twenty-four hours to give time for the separation of the serum, which is then transferred to smaller receptacles for preservation.

The dose of serum prepared in this way, when used to protect from diphtheria infection, is 5 c.c. for a child under ten years of age, and 10 c.c. for older children. This does not afford an absolute protection, but is believed to be generally effective, and in case of failure the attack is said to be of a mild character. The curative dose of Roux's serum is 20 c.c. for children, and 30 to 40 c.c. for patients over fifteen years of age. The larger dose is divided and given, at the same time, by subcutaneous injection in two places. Antiseptic precautions are taken in giving these injections, and a little absorbent cotton is placed over the puncture.

Results of Treatment with Serum of Immune Animals.

We take the following statistics from a recent (December, 1894) paper by Woodhead:

"It may be pointed out from statistics that no fewer than 13,694 cases were notified in London during the year 1893. Among these cases there was a mortality of 3,195 or 23.3 per cent. (see *The Lancet* corrected statistics). In a series of most carefully prepared and tabulated statistics, those of the Metropolitan Asylums Board's hospitals which receive diphtheria patients

TABLE I.—*Metropolitan Asylums Board: Admissions and Case Mortality, Diphtheria*, 1888–93.

YEAR.	No. of Admissions.	No. of Deaths.	Percentage of Case Mortality.
1888	99	46	46.4
1889	722	275	38.0
1890	942	316	33.5
1891	1312	397	30.2
1892	2009	583	29.0
1893	2848	865	30.3

NOTE.—Diphtheria cases have only been admitted into the hospitals since October 23, 1888.

TABLE II.—*Showing the Mortality at Various Ages from Diphtheria admitted into the Metropolitan Asylums Board's Hospitals in the Years* 1888–93.

AGES.	Cases Admitted.	Died.	Mortality per cent.
Under 1	146	102	69.9
1 to 2	447	291	65.1
2 to 3	639	388	60.7
3 to 4	826	416	50.4
4 to 5	913	400	43.8
Totals under 5	2,971	1,597	53.8
5 to 10	2,462	705	28.6
10 to 15	885	93	10.5
15 to 20	588	23	3.9
20 to 25	426	22	5.2
25 to 30	269	13	4.8
30 to 35	137	5	3.6
35 to 40	87	5	5.7
40 to 45	50	6	16.8
45 to 50	27	4	
50 to 55	13	4	
55 to 60	11	1	
And upward	6	3	
Totals	7,932	2,481	31.3

TABLE III.—*Serum Treatment of Diphtheria.*

		Number of Cases.	Number of Deaths.	Percentage of Mortality.	Percentage of Previous Mortality.
Roux, Martin, and Chaillou.	Paris	448	109	24.5	51.7‡
Ehrlich, Kossel, and Wassermann.....	Berlin.....	220	52	23.6	34.7§
*Canon.....	"	15	3	20.0	25.0
*Schubert.....	"	34	6	18.0	
*Voswinkel.....	"	60	19	33.3	46.5
Ehrlich	"	89	12	12.3	
†Kossel.....	"	233	54	23.0	
*Korte	"	121	40	33.1	53.8
Bokai.....	Budapest.....	35	5	14.2	53.8
Heubner.....	.....	96	37	38.5	62.5
Katz	Berlin	128	17	13.2	38.9‖
Aronson	"	255	31	12.1	32.5-41.7
Ranke and Oertel.	.....	19	4	21.0	
The Lancet	Austrian Riviera	70	7	10.0	
Weibgen	Berlin	65	18	28.8	40.0
Muehleck	Philadelphia....	2	..	..¶	
Dr. White.....	Will'd Parker Hospital, New York...	32	8	25.0	42.7
Cases reported in *The Lancet* and *British Medical Journal*.....		79	9	11.4	

* Canon, Schubert, and Voswinkel's statistics, and probably also the bulk of Korte's, are really included in the Ehrlich, Kossel, and Wassermann's table.

† Kossel's statistics appear to be simply an extension of those given by Ehrlich, Kossel, and Wassermann.

‡ Mortality in Trousseau Hospital at same period without use of serum 63.2 per cent.

§ Based on previous seven years' statistics.

‖ Based on three years' statistics. Last year's mortality was 41.7 per cent.

¶ Two not treated died.

(Table I.) we find that during the year 1893 the case mortality among cases of diphtheria admitted to the wards of these hospitals was a little over thirty per cent.—under conditions which we may say are practically unknown in France and in most parts of Germany and Austria. This is sufficiently startling, but if we examine earlier statis-

tics we find that during the years 1888–90 the case mortality, as indicated by the admissions and deaths, was much higher. On reference to Table I. it will be seen that since the year 1888 the case death-rate has fallen from 46.4 to 30.3 per cent., although the number of cases of diphtheria treated has risen enormously. The mortality from diphtheria in London during the same period has also risen very considerably. The case mortality at different ages is brought out on reference to Table II. Comparing these results of treatment in our hospitals with those obtained in the Paris and Berlin hospitals (see Table III.), the records of which are published, it is at once seen how much more favorable are the results obtained in the hospitals of the Metropolitan Asylums Board. It will be noted, however, that the figures applying to the earlier years represented a case mortality almost as high as obtained in the French and German hospitals immediately before the antitoxic-serum treatment was introduced. The mortality in hospitals must always be greater than in private practice from the fact that so many of the cases are so serious; some of the patients, indeed, are actually moribund when admitted."

Fischer (January, 1895) reports 34 cases—30 malignant and 4 mild—treated with a mortality of two = 5.8 per cent. He says:

"These cases were not selected, for some were poorly nourished, some in excellent vitality, with careful nursing and good hygiene. The main point was to apply the antitoxin as early as possible, and counteract the septic matter absorbed, and thereby avoid complications, besides using the local treatment of swabbing the throat with a 1 to 2,000 bichloride of mercury solution, using a fresh swab for each application, and burning the same immediately after using it.

"The technique of injection is simple. Having properly sterilized the syringe by boiling and using 0.5 per

cent. tricresol, I commence by injecting 10 c.c. in mild cases, and 20 c.c. in malignant cases, by pinching a fold of the skin in the intrascapular region, and allowing the serum to be slowly injected. I believe it proper, however, to have a syringe of suitable size and inject the required amount, rather than inject several places. The calibre of the latter must necessarily be quite large, owing to the thickness of the serum, which is at times rather mucilaginous. It is proper to note all differences and effects on the false membrane and the swelling of the glands, the behavior of the temperature, the condition of the urine, the effect on the heart, and especially the pulse.

"There should be no hesitation in injecting on the second day, and, if no effect is seen, repeating the injection on the third day, as there is absolutely no risk from the injection. It is a perfectly safe remedy, and shows no immediate reaction. It differs from tuberculin and vaccine in that it causes no reaction. A case of antitoxin treatment will show no symptoms directly attributable to the remedy, unless it be in some cases of urticaria. The temperature does not fall by crisis, but by lysis, with antitoxin treatment. Massage of the serum after the injection should not be practised, according to Heubner, Aronson, Baginsky, and others."

The efficacy of the antitoxin treatment has been combatted in Berlin by Hansemann and others. The opponents of the treatment point to a considerable number of recorded cases in which relapses have occurred after apparent recovery following treatment with the antiseptic serum; also to the occurrence of diphtheria in children who had been treated with immunizing doses of the serum; and to the failure to cure in a considerable number of cases notwithstanding the administration of large doses of the antitoxic serum. All of these facts must be admitted, but the experimental (on animals) and clinical

evidence heretofore submitted appears to establish the value of the treatment when applied before the disease has progressed too far. It must be remembered that the antitoxin has no power to destroy the diphtheria bacilli, or to relieve the suffocation resulting from obstruction of the larynx, or to cure an acute parenchymatous nephritis due to the action of the deadly toxin elaborated by the Klebs-Löffler bacillus.

Saltmann (1895) reports a mortality of 39.8 per cent. in 71 cases treated in the children's hospital at Leipsic before the use of the antitoxin. The report relates to cases treated from April 1, to December 31, 1894. During the last five months of this period 122 cases were treated and most of them received the antitoxin. The mortality was 18 per cent.

In an article contributed to *McClure's Magazine* for March, 1895, Dr. Hermann Biggs, of New York, says: "That prepared in this country under the supervision of the New York City Health Department has, at the time of writing, been already employed in more than one hundred and fifty cases, and the mortality in the cases thus treated has been about twelve per cent."

Klebs has proposed to treat diphtheria with a preparation obtained from cultures of the diphtheria bacillus which he calls antidiptherin. The clinical experiments made by Vulpius and by Zappert show that the treatment has no value and it has been abandoned.

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V.

FOOT-AND-MOUTH DISEASE.

This is an infectious disease of cattle, sheep, goats, and swine, the etiology of which, so far as the specific infectious agent is concerned, has not been determined. Schottelius has (1892) described a microörganism found by him in the contents of the vesicles of foot-and-mouth disease which seems to be peculiar to this disease, but he was not able to demonstrate its etiological relation by inoculation experiments. The microörganism referred to is a streptococcus so far as its morphology is concerned, but differs from the previously known streptococci in being extremely motile.

The extent to which the disease in question prevails in some parts of Europe is shown by the statistics for 1891 of the prevalence of this disease in Germany. According to the Reichsseuchenbericht it prevailed most extensively in the southern portion of Germany. The total number of infected farms was 47,865; the total number of infected cattle was 394,640; of sheep, 240,904; of goats, 3,378; of swnie, 182,208. Behla (1892) has made inoculation experiments with the filtered saliva of infected cattle to which he added one to two per cent. of carbolic acid, and claims to have produced immunity in young pigs and lambs. The duration of immunity is not, however, very long even in animals which have recovered from an attack of the disease—said to be from six months to three

years—and a practical method of restricting the disease by means of protective inoculations has not as yet been introduced.

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VI.

GLANDERS.

The etiological relation of the bacillus discovered by Löffler and Schütz, in 1882, to glanders is well established. Experiments by the bacteriologists named, and frequently repeated by others, show that pure cultures of this bacillus injected into horses, asses, and other susceptible animals, produce genuine glanders. The field-mouse and the guinea-pig are especially susceptible to infection by experimental inoculations; the cat and the goat may also be infected; rabbits, sheep, and dogs are but slightly susceptible, and swine, cattle, white mice, and house-mice are immune.

The toxic substances produced in cultures of the glanders bacillus when concentrated in the form of a glycerin extract constitute the so-called malleïn, which has been extensively used in the diagnosis of glanders in horses. As is the case when animals infected with tuberculosis are inoculated with tuberculin, animals infected with glanders have a decided rise of temperature after receiving a sufficient dose of malleïn beneath the skin.

Babes (1892) reports that the toxic substance in cultures of the glanders bacillus may be obtained by precipitation with alcohol; and that malleïn obtained from filtered cultures to which glycerin has been added, or the alcoholic precipitate, may be successfully used for protecting susceptible animals against glanders infection or

for curing the disease after infection. He has demonstrated the therapeutic value upon guinea-pigs and upon two horses which are said to have been cured of chronic glanders. When large and repeated doses are injected into healthy animals they produce nephritis and general marasmus. The action upon horses infected with glanders is very marked and small doses may even cause death.

Kresling (1892) recommends potato cultures as preferable to bouillon cultures for the preparation of malleïn. The potatoes are to be washed, before sterilization, in a five per cent. bicarbonate of soda solution, "until the wash-water remains clear." They are then cooked for an hour and twenty minutes. After planting upon the surface glanders bacilli from a previous culture they are placed in an incubator at 36° to 36.5° C., with provision to prevent them from becoming dry. At the end of two weeks the growth is removed with a platinum spatula and added to nine parts of water, in which it is well mixed by rubbing. It is then allowed to stand for twenty-four hours, after which it is sterilized for fifteen minutes at 110° C. (a lower temperature would no doubt answer quite as well). After cooling it is passed through a Chamberlain filter by means of a pressure of six atmospheres. The filtrate is then carefully evaporated over a water-bath to one-fourth its volume, and to this concentrated extract glycerin is added in the proportion of one part to two. The mixture is again sterilized in the autoclave at 110° C. When injected into healthy horses in the dose of 2 c.c. this malleïn does not cause an elevation of temperature exceeding 0.5° to 0.8° C. But 1 c.c. injected into a horse having glanders causes its temperature to mount to 40° C., and at the point of inoculation a

considerable swelling is developed which lasts from four to six days—in healthy horses a swelling the size of a man's fist is developed at the point of inoculation, which disappears within twenty-four hours.

In Pasteur's laboratory, according to Nocard (1892), malleïn is prepared as follows: The glanders bacillus is first made so virulent by successive inoculations in susceptible animals that it will kill a rabbit or a white mouse in a few hours. This virulent bacillus is cultivated in glycerin-pepton-flesh-infusion (five per cent. of glycerin and five per cent. of pepton). The cultures are kept in the incubating oven for four weeks at a temperature of 31° C., and then sterilized in the autoclave at 110° C. They are then filtered through paper and evaporated, in vacuo, over sulphuric acid, at a low temperature, to one-tenth of the original volume. The result is a sirup-like, dark brown, strong-smelling liquid, which is about one-half glycerin. This can be preserved in a cool and dark place for a long time. When it is to be used nine parts of a 0.5 per cent. solution of carbolic acid are added to one part of the glycerin extract. The concentrated extract, when injected into a healthy horse in the dose of 0.5 to 1 c.c., causes a local swelling which disappears after two or three days. The temperature of the body is elevated from 1.5° to 2° C. as a result of the injection, and there is chilliness, loss of appetite, and debility. When the diluted malleïn is injected in healthy animals in the dose of 2.5 c.c. no reaction occurs. On the other hand, this dose causes an intense febrile reaction in horses with glanders. There is a chill followed by an elevation of temperature amounting to 2° to 3° C., accompanied by dyspnœa and great debility; in some cases the animal died as a result of the inoculation.

For the preparation of the active substance in a dry condition, Foth gives the following directions: The cultures are evaporated at a temperature not exceeding 80° C. to one-tenth of their volume, and filtered. The clear and thick, dark brown liquid is then slowly dropped into absolute alcohol (25 to 30 parts) with constant stirring. A flaky, white precipitate is thrown down, and accumulates as a pale yellow mass upon the sides and bottom of the vessel. After standing for twenty-four hours the alcohol is carefully drawn off and the precipitate washed with absolute alcohol. This is to be carefully done, and to avoid loss will require several days. The precipitate is then placed upon a thick paper filter and thoroughly washed by drawing alcohol through it by means of an exhaustion apparatus, after which the purified precipitate is collected and dried with care at a low temperature —best in a vacuum over sulphuric acid. A spongy, crumbling mass is thus obtained, which is easily crushed to form an extremely light white powder. This is readily soluble in water. It is not at all hygroscopic, and can be preserved in a dry condition without difficulty. The dose for a horse is 0.1 gm.

De Schweinitz and Kilborne, in a paper published in November, 1892, state that in December, 1890, they

"Extracted from culture liquids of the bacillus malleus an albumose which appeared to be the active principle in these cultures. At that time a preliminary experiment was conducted to see if this substance could be used to make guinea-pigs immune to the disease—glanders. The result was that out of a set of five, three vaccinated and two checks, only one, a vaccinated animal, recovered from an inoculation of a glanders culture. This experiment has since been repeated with sets of ten

and twelve guinea-pigs each, with, at present writing, only negative results. A note of this work was published in the 'Annual Report of the Department of Agriculture for 1891.' The albumose was best obtained from the cultures, after the removal of the germ, by means of a Pasteur filter, by precipitation with absolute alcohol, resolution in water, and reprecipitation."

Babes (1892) claims to have succeeded in immunizing guinea-pigs against glanders by means of the toxic substances contained in cultures of the bacillus.

In a recent paper (1894) Foth has reported the results of extended experiments which have been made with his "Malleïnum siccum" in Austro-Hungary. These results are stated as follows:

The experiments were for the most part made by Professor Schindelka, of Vienna. The tests were made with doses ranging from 0.01 gm. to 0.02 gm. The number of horses treated, for diagnostic purposes, was 455; of these 147 were examined post-mortem. In general the infected horses reacted and the others did not. A reaction of 2° C. and upward, running a typical course, was evidence that the animal was infected, and such animals were killed and carefully examined by autopsy.

A reaction of 1.3° to 1.9° C., running a typical course, was taken as evidence that the animal was probably infected, and called for its isolation and a subsequent inoculation after an interval of four weeks.

A reaction of less than 1.2° C., or an atypical course of the febrile reaction, was taken as evidence of non-infection.

The typical febrile reaction consisted in a rapid or gradual elevation, according to the dose, then a fall of some tenths of a degree, a subsequent elevation to the highest previously reached point or above, and a gradual

fall to the normal. The atypical reaction, which sometimes occurs in healthy animals, consists in an early and rapid elevation followed by an equally rapid fall to the normal. To properly distinguish the typical temperature curve, upon which the diagnosis depends, hourly observations are considered necessary.

Schütz (1894), as a result of his experiments on fifty-four horses, arrives at the conclusion that malleïn may give rise to the so-called "typical reaction" in horses which are not infected with glanders.

Hutyra and Preiz (1894), as a result of their extended researches, arrive at the conclusion that the use of malleïn constitutes the most important means for the early diagnosis of glanders in horses. They conclude that a temperature of 39.4° C. may be accepted as a safe positive malleïn reaction. According to them the reaction commences from four to six hours after the injection, and reaches its maximum in from eight to fourteen hours—rarely in sixteen to twenty hours. The return to the normal occurs in from twenty-four to thirty-six hours. The authors last named give the following directions for the preparation of malleïn: The virulence of the glanders bacillus is first increased by passing it through a series of guinea-pigs. Cultures are then made upon sterilized potato. When the culture and potato have become quite dry and dark colored they are collected in a glass dish and covered with a liquid consisting of equal parts of glycerin and distilled water, containing 3 to 5 parts per thousand of mercuric chloride. After standing for from ten to fourteen days in an incubating oven at 37.5° C., the liquid is filtered through paper and sterilized for an hour in a steam-sterilizer. This liquid remains sterile on account of the presence of mercuric chloride, and may be preserved a

long time without losing its activity. The dose is from 0.3 to 0.5 c.c., which is diluted to 3 c.c. with carbolic acid water (0.5 per cent. solution). The diluted solution may also be kept a long time without losing its activity.

Bonome and Vivaldi (1892) have tested the action of malleïn obtained by precipitation with alcohol upon various animals. Guinea-pigs were found to resist comparatively large doses (10 to 15 milligr.), while rabbits and cats were more sensitive to the toxic action. In guinea-pigs and rabbits infected with glanders bacilli very small doses had a favorable influence upon the progress of the infection, and in healthy guinea-pigs a certain degree of immunity was induced by the repeated injection of small doses.

In a subsequent paper (1894) Bonome reports that he has had favorable results in the treatment of chronic glanders in man by doses of $\frac{1}{15}$ to $\frac{1}{20}$ c.c. The first dose is said to have caused an elevation of temperature, headache, polyuria, etc., but upon repeating the dose after two or three days a decided improvement of the general symptoms followed.

Chenot and Picq (1892) claim to have cured glanders in guinea-pigs by injections of blood-serum from the ox, which animal has an immunity from the disease. They also state that the blood-serum of the ox is germicidal for the glanders bacillus. Guinea-pigs treated with ox-serum, either before or after infection, recovered in seven cases out of ten. When inoculated with very virulent cultures, which usually killed these animals in five days, the animals are said to have survived from twenty-one to forty-two days.

Bonome (1894) reports his success in curing infected guinea-pigs by means of filtered cultures made in the

blood-serum of the ox. He was not, however, successful in accomplishing this result with malleïn made in the usual way.

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VII.

HOG CHOLERA.

Researches made by the bacteriologists connected with the Agricultural Department (Bureau of Animal Industry) have established the fact that two distinct diseases of swine, due to different bacilli (one motile and the other not) prevail in epidemic form in various parts of the United States. The differentiation of the bacillus of hog cholera was made in 1885, and a report by Theobald Smith, relating to this infectious disease of swine, has recently been published (1894). The bacillus produces in swine a disease in which the principal pathological lesions are found in the large intestine, the mucous membrane of which is more or less ulcerated and necrosed; in some cases there is at the same time a pneumonia of limited extent.

The bacillus kills rabbits, mice, and guinea-pigs when injected subcutaneously or introduced into the stomach. A small dose is fatal to a rabbit in about seven days, but larger doses kill in a shorter time, and it is more promptly fatal when injected into the circulation. Several varieties of this bacillus have been encountered by the bacteriologists of the Agricultural Department; these differ chiefly in pathogenic virulence, and the curious fact is stated that the variety first studied, after artificial cultivation for six and a half years, has not lost its pathogenic power. It still kills rabbits when injected into the

circulation in small amounts (0.12 c.c.). All of the varieties closely resemble the well-known *Bacillus coli communis*, which has its normal habitat in the intestine of healthy men and animals. According to Smith the bacillus (*B. typhi murium*) discovered by Löffler in 1890, which causes a fatal infectious disease in mice, is nearly allied to the hog-cholera bacillus, and should be included in the same group — which may be denominated the "colon group."

The experiments thus far made with reference to protective inoculations against hog cholera have not given very satisfactory results. Selander and Metchnikoff have reported success in immunizing rabbits, but according to Smith their experiments were made with the bacillus of swine plague, and not with that of hog cholera as they supposed. The following conclusions have been formulated by Smith as a result of his extended experiments:

"1. It is possible to produce immunity toward hog-cholera and swine-plague bacteria in the very susceptible rabbit and the less susceptible guinea-pig. In the rabbit the only promising method of immunization toward hog cholera is the use of gradually augmented doses of attenuated cultures.

"2. Immunization toward swine-plague is produced artificially with much greater ease than toward hog-cholera bacteria.

"3. The blood-serum of animals protected against hog cholera and swine plague is almost as efficacious in producing immunity soon after treatment as the bacterial products obtained from cultures.

"4. Different degrees of immunity in both hog cholera and swine plague lead to different forms of the inoculation disease. The greater the immunity short of complete protection the more prolonged and chronic the disease induced subsequently by inoculation.

"5. Pathogenic bacteria may remain in the organs of inoculated animals some time after apparently full recovery. Their presence may or may not be associated with lesions recognizable by the naked eye.

"6. The toxicity of sterilized cultures appears to be directly proportional to the number of bacteria in the injected fluid."

The experiments of Moore, reported in Bulletin No. 6 of the Bureau of Animal Industry, show that the bacillus of hog cholera does not become attenuated by being passed through rabbits, and that in the experiments of Metchnikoff, which led him to conclude that this is the case, the bacillus of swine-plague, and not that of hog cholera, was used.

De Schweinitz has studied the chemical products of the hog-cholera bacillus (1890) and has obtained from the cultures cadaverin, methylamin, a ptomaïne ("sucholotoxin"), and an albumose ("sucholoalbumin").

Novy (1890) has also obtained, by Brieger's method, a basic toxic substance ("susotoxin") which kills rats in the dose of 0.125 to 0.25 c.c. He also obtained from concentrated cultures, by precipitation with absolute alcohol, a toxalbumin which, when dried, killed rats in three or four hours in the dose of 0.05 to 0.01 gm.

Both of the authors named obtained experimental evidence indicating that these toxic substances obtained from cultures, when given to susceptible animals in nonlethal doses, cause them to be immune from the pathogenic action of small quantities of a culture of virulent hog-cholera bacilli.

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VIII.

HOG ERYSIPELAS.

The disease known in Germany as *Rothlauf*, and in France as *rouget*, is due to a slender bacillus, which is, apparently, identical with Koch's bacillus of mouse septicæmia, which he first obtained by inoculating mice with putrefying blood or flesh infusion. The bacillus of hog erysipelas (*Rothlauf*) was first accurately described by Löffler and Schutz (1885), although Pasteur had previously ascribed the etiology of this disease to a minute bacillus —no doubt the same—and had proved the practicability of protecting swine from the ravages of the disease by inoculations with an attenuated virus. This bacillus is pathogenic for swine, rabbits, white-mice, house-mice, pigeons, and sparrows. Guinea-pigs, field-mice, and chickens are immune. Swine become infected by the ingestion of food containing the bacillus, and usually die within two or three days.

Pasteur's first studies relating to the etiology of "*rouget*" were made, in collaboration with Chamberland, Roux, and Thuillier, in 1882. Pasteur found that the virulence of his cultures was increased by passing them through pigeons and diminished by passing them through rabbits. By a series of inoculations in rabbits he obtained an attenuated virus suitable for protective inoculations in swine. In practice he recommended the use of a mild virus first, and after an interval of

twelve days of a stronger virus. These inoculations have been extensively practised in France, and the fact that immunity may be established in this way is well demonstrated. There has been some doubt, however, as to the practical value of the method, as its application has been attended with some loss, and there appears to be danger that the disease may be spread by the alvine discharges of inoculated animals. In a region where the annual losses from the disease are considerable, and where the soil is, perhaps, thoroughly infected with the bacilli, protective inoculations probably afford the best security against loss. But when it is practicable to stamp out the disease by quarantine of infected animals, disinfection of localities in which cases have occurred, and strict attention to cleanliness, this will probably be found the best method of combating the malady.

In a recent paper (1894) Chamberland states that in the last seven years, during which time protective inoculations have been practised in France on a large scale, the mortality from rouget has been reduced to 1.45 per cent., whereas before these inoculations were practised the mortality from this disease was about twenty per cent. Losses amounting in some instances to as much as ten per cent. have resulted from the inoculations. These are ascribed by Chamberland to secondary infection, through the inoculation wound, with other pathogenic bacteria.

Jakobi (1888) reports the results of inoculations made in 1887 and 1888 with "vaccines" obtained from Pasteur's agent in Paris. His results agree with those previously reported by Lydtin in showing a smaller loss, as a result of the inoculations, among young pigs than among older ones—over sixteen weeks. The loss among young pigs

was only 1.3 per cent. The animals which survived subsequently escaped infection, while others not inoculated, associated with them, succumbed to the disease.

Hutyra has given the following statistics of inoculations made in Hungary during the year 1889, with "vaccines" obtained from the Pasteur laboratory in Vienna: 48,637 pigs were inoculated on 117 different farms. Of these 143 (0.29 per cent.) died between the first and second inoculation. After the second inoculation 59 animals died (0.1 per cent.). During the year following the inoculations 1,082 inoculated pigs died of *Rothlauf*. Before the inoculations the annual loss in the same localities is said to have been from ten to thirty per cent. Upon one farm 220 pigs which had been inoculated were associated with 1,500 not inoculated. The loss among the latter was fifty per cent., among the former 2.27 per cent.

In a later communication (1894) Jakobi gives the following results of inoculations made since by the same method: 1889, inoculated 133, loss, 5; 1890, inoculated 151, loss, 2; 1891, inoculated 158, loss, 0; 1893, inoculated 223, loss, 0; 1894, inoculated 145, loss, 4. Total inoculated, 1,036; total loss, 14. These inoculations were made upon 19 different farms, and principally upon pigs less than four months old. The inoculated pigs were isolated to prevent the communication of the disease to other unprotected pigs.

Inoculations with Blood-serum of Immune Animals.

The experiments of Lorenz, commenced in 1891, seem to establish the fact that there is an antitoxin in the blood of animals which have an acquired immunity against this disease which may be used for producing

immunity in other animals, or for the cure of the disease in animals already infected. In his latest communication (1894) Lorenz says :

" When I read in the journals of the discovery of Behring and Kitasato that the blood of animals immunized against tetanus, when injected beneath the skin of other animals, gave them an immunity against tetanus, I had in my possession rabbits which were immunized against *Rothlauf*. I took from one of these some blood from the ear vein, injected it under the skin of a mouse, inoculated this later with a *Rothlauf* culture, and made the discovery, in this and a series of subsequent experiments, that the blood of an animal immune against *Rothlauf* contains an immunizing substance. I further ascertained that this substance is found only in the blood-serum, and not in the solid portions of the body organs, etc., and with the exception of the blood was found only in the secretions of serous membranes. I also found that the immunizing substance is only to be found for a certain time after renewed infection in the immune animals, and that it gradually disappears, without the loss of immunity in the animal, however. Finally, I discovered that the animals into which one injects blood-serum from immune animals do not acquire a lasting immunity, but are only immune for a relatively short time."

In experiments made in 1893 and 1894, with a view to producing immunizing serum for protective inoculations on a large scale, Lorenz met with some disappointments ; but he proposes to renew his attempts and hopes to avoid the difficulties which have been brought to light by experience, one of which he states as follows :

" When an animal already immunized against *Rothlauf* receives an injection of a considerable quantity of a culture of the bacillus, in order to cause the production in

its blood of a serum of high therapeutic value, the animal bears these injections without any notable reaction. But its blood-serum contains during the following days, besides the immunizing substance, also poisonous substances, and blood which is taken too soon (twenty-four hours) after the injection has a toxic action upon animals which are already infected. If this poisonous serum is injected into a mouse which has been infected two days before with *Rothlauf* bacilli, in the dose of about 0.05 c.c., death occurs in a few hours, even when scarcely any evidence of sickness had been observed before the injection."

The fact that mice infected with this bacillus may be cured by injecting into them blood-serum from an immunized rabbit has also been demonstrated by F. Klemperer (1892). In his experiments with the bacillus of mouse septicæmia, and with Friedländer's bacillus, he found that serum from an immune rabbit may be used to immunize mice and also to cure them after infection, while serum from a non-immune rabbit has no such action. The immunity produced in this way was found to be specific. That is, animals immunized against the pathogenic action of one of these bacilli were not protected against infection by the other. The "*heilserum*" when added to cultures *in vitro* did not prove to have any special bactericidal action.

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IX.

HYDROPHOBIA.

Notwithstanding the extended researches made, especially in Pasteur's laboratory, the etiology of hydrophobia still remains unsettled. It has been demonstrated by experiment that the virus of the disease is located in the brain, spinal marrow, and nerves of animals which have succumbed to the disease, as well as in the salivary secretions of rabid animals; and that the disease may be transmitted by intravenous inoculation, or by introducing a small quantity of virus beneath the dura mater, with greater certainty than by subcutaneous inoculations. But the exact nature of this virus has not been determined. The fact that a considerable interval elapses after inoculation before the first symptoms are developed indicates that there is a multiplication of the virus in the body of the infected animal; and this is further shown by the fact that after death the entire brain and spinal marrow of the animal have a virulence equal to that of the material with which it was inoculated in the first instance. The writer's experiments (1887) show that this virulence is neutralized by a temperature of 60° C., maintained for ten minutes—a temperature which is fatal to all known pathogenic bacteria in the absence of spores. But recent experiments show that certain toxic products of bacterial growth are destroyed by the same temperature. We are, therefore, not justified in assum-

ing that the morbid phenomena are directly due to the presence of a living microörganism; and, indeed, it seems probable, from what we already know, that the symptoms developed and the death of the animal are due to the action of a potent chemical poison of the class known as toxalbumins. But, if this is true, we have still to account for the production of the toxic albuminoid substance, and, in the present state of knowledge, have no other way to explain its increase in the body of the infected animal than the supposition that a specific, living germ is present in the virulent material, the introduction of which into the body of a susceptible animal gives rise to the morbid phenomena characterizing an attack of rabies.

Pasteur and his associates have thus far failed to demonstrate the presence of microörganisms in the virulent tissues of animals which have succumbed to an attack of rabies. Babes has obtained micrococci in cultures from the brain and spinal cord of rabid animals, and states in his article on hydrophobia in "*Les Bacteries*" (second edition, p. 791) that pure cultures of the second and third generation induced rabies in susceptible animals; but his own later researches do not appear to have established the etiological relation of this micrococcus.

Gibier (1884) has reported the presence of spherical refractive granules, resembling micrococci, in the brain of rabid animals, which he demonstrated by rubbing up a little of the cerebral substance with distilled water. As these supposed micrococci did not stain with the usual aniline colors and were not cultivated, it appears very doubtful whether the refractive granules seen were really microörganisms.

Fol (1885) claims to have demonstrated the presence of minute cocci, 0.2 μ in diameter, in sections of spinal cord from rabid animals, by Weigert's method of staining. The cords were hardened in a solution of bichromate of potash and sulphate of copper, colored with a solution of hæmatoxylon, and decolorized in a solution of ferrocyanide of potash and borax.

The writer (1887) has made similar preparations, carefully following the method as described by Fol, but was not able to demonstrate the presence of micro-örganisms in the numerous sections made. Nor have the observations of Fol been confirmed by the researches of other bacteriologists who have given their attention to the subject since the publication of his paper.

Pasteur first announced his success in reproducing rabies in susceptible animals by inoculations of material "from the medulla oblongata, the frontal lobes of the cerebral hemispheres, and the cerebro-spinal fluid" in a communication to the Academy of Sciences made on May 30, 1881. At the same time he reported his success in the discovery of "a method for considerably shortening the period of incubation in rabies, and also of reproducing the disease with certainty." This was by inoculations, made after trephining, upon the surface of the brain with material obtained from the brain of a rabid animal. Dogs inoculated in this way developed rabies in the course of two weeks, and died before the end of the third week—sometimes of furious rabies and sometimes of the paralytic form of the disease. In a second communication (December 11, 1882) Pasteur reports his success in communicating the disease by the intravenous injection of virus from the central nervous system; also the ex-

perimental demonstration of the fact that all forms of rabies may be produced by the same virus; also that all portions of the spinal cord of rabid animals are virulent, as well as all parts of the brain; also that an animal (dog) which had recovered from a mild attack after inoculation proved to be subsequently immune, and that "this observation constitutes a first step toward the discovery of the prophylaxis of rabies." On February 25, 1884, many important facts are stated which had been developed during the continuous study of the disease, and among others the fact that by passing the virus through a series of animals of the same species a fixed degree of virulence is established, for each susceptible species, as shown by a definite and uniform period of incubation. By this method a virus had been obtained which produced rabies in rabbits in seven or eight days, and another which caused the development of the disease in guinea-pigs in five or six days after inoculation. In a subsequent communication (May 19, 1884) evidence is given to show that by successive inoculations in monkeys the period of incubation is prolonged, and that the attenuated virus obtained from a monkey, after several successive inoculations in this animal, when inoculated into the dog, no longer produces fatal rabies; and that dogs so treated are subsequently immune.

In his address before the International Medical Congress at Copenhagen (August 11, 1884), after a review of the facts developed during his experimental researches made during the preceding four years, Pasteur gives an account of the test made by a commission, appointed by the Minister of Public Instruction, to determine the efficacy of his method as applied to the protection of dogs. He says that he gave to the commission nineteen dogs

which had been rendered refractory against rabies by preventive inoculations. These nineteen dogs and nineteen control animals, obtained from the pound without any selection, were tested at the same time. The test was made upon some of the animals of both series by inoculation with virulent material upon the surface of the brain, and upon others by allowing them to be bitten by rabid dogs, and upon still others by intravenous inoculations. Not one of the protected animals developed rabies; on the other hand, three of the control dogs out of six bitten by a mad dog developed the disease, five out of seven which received intravenous inoculations died of rabies, and five which were trephined and inoculated on the surface of the brain died of the same disease. In a subsequent report the commission, of which M. Bouley was president, stated that twenty-three protected dogs which were bitten by ordinary mad dogs all remained in perfect health, while sixty-six per cent. of the control animals, bitten in the same way, developed rabies within two months.

In his communication of October 26, 1885, Pasteur reports his discovery of the fact that the virulence of the spinal cord of a rabbit is gradually attenuated by hanging it in a dry atmosphere, and is finally entirely lost; also that he had been able to make a practical application of this discovery in the protection of dogs by means of successive inoculations beneath the skin of an emulsion of spinal marrow attenuated in this way. The first inoculation was to be made with a portion of spinal cord which had been kept long enough to deprive it of all virulence, and this was followed by daily inoculations with more virulent material until, finally, material was used from a cord only a day or two old.

With reference to his first inoculations in man, Pasteur says:

"Making use of this method, I had already made fifty dogs of various races and ages immune to rabies, and had not met with a single failure, when, on the 6th of July, quite unexpectedly, three persons, residents of Alsace, presented themselves at my laboratory."

These persons were Theodore Vone, who had been bitten on the arm on July 4th, Joseph Meister, aged nine, bitten on the same day by the same rabid dog, and the mother of Meister, who had not been bitten. The child had been thrown down by the dog and bitten upon the hand, the legs, and the thighs, in all in fourteen different places. Pasteur commenced the treatment on July 6th, by injecting beneath the skin of this child an emulsion of cord which had been kept for fourteen days; this was followed by twelve more inoculations made on successive days with cord of increasing degrees of virulence—the last with cord a day old. On March 1, 1886, Pasteur reported to the Academy of Sciences the fact that the boy Meister remained in good health and gave detailed information with reference to a number of cases which had since been treated by the same method.

With reference to the duration of the immunity resulting from these inoculations Pasteur says (1886) that out of fourteen dogs inoculated with "ordinary street virus," by trephining, at the expiration of a year after the protective inoculations had been practised, eleven resisted; out of six tested in the same way at the end of two years two proved to be immune.

In November, 1886, Pasteur communicated to the Academy of Sciences the results of his experiments with refer-

ence to a modification of his method as at first employed —the so-called intensive method. This modification consisted in making the inoculations with cords of increasing virulence in more rapid succession.

The method followed at Odessa, as reported by Gamaleïa (1887), is shown below, the day being given above and age of the cord below.

$$\frac{1}{14\text{–}13},\ \frac{2}{12\text{–}11},\ \frac{3}{10\text{–}9},\ \frac{4}{8\text{–}7},\ \frac{5}{6\text{–}5},\ \frac{6}{4\text{–}3},\ \frac{7}{2\text{–}10},\ \frac{8}{8\text{–}6},\ \frac{9}{4},\ \frac{10}{2}.$$

Since the adoption of this method and the use of larger quantities of virus, according to Gamaleïa, there have been no deaths among those inoculated, numbering more than two hundred at the time the report was made. The author last referred to concludes from his experience that "the mortality diminishes in direct relation to the quantity of the vaccine injected."

Bujwid (1889) reports a total of 670 inoculations, with 9 deaths, made at Varsovie during the years 1886, 1887, and 1888. His method is shown below.

$$\frac{1}{12\text{–}10},\ \frac{2}{8\text{–}6},\ \frac{3}{4},\ \frac{4}{3},\ \frac{5}{6},\ \frac{6}{4},\ \frac{7}{3}.$$

The results of inoculations made at the Pasteur Institute in Paris during the years 1886 to 1890 are given in the following table :

Year.	Number Treated.	Died.	Mortality.
1886	2,671	25	0.94
1887	1,770	13	0.73
1888	1,622	9	0.55
1889	1,830	6	0.33
1890	1,540	5	0.32
Total	9,433	58	0.61

In the following table, A includes all persons treated who had been bitten by an animal proved to be rabid; B, persons bitten by animals examined by veterinary surgeons and pronounced rabid; C, persons bitten by animals suspected of being rabid. The figures relate to the year 1890:

	Number Treated.	Died.	Mortality.
A	416	0	....
B	909	4	0.44
C	215	1	0.46

Bordoni-Uffreduzzi gives the following statistics with reference to the inoculations practised at the Pasteur Institute in Turin during the years 1886 to 1891: 81 persons were inoculated by the method first proposed by Pasteur, with a mortality of 2.46 per cent.; 925 persons were subsequently inoculated by the same method, but with larger doses of virus, with a mortality of 1.72 per cent. Finally, 338 persons were inoculated with still larger doses, with a mortality of 0.29 per cent.

At the Pasteur Institute in Palermo the number of persons inoculated in the four years prior to 1891 was 662, with a mortality among the inoculated of 0.6 per cent. In Bologna (1890) 210 persons bitten by dogs undoubtedly mad were inoculated, with a mortality of 0.47 per cent.

In the Pasteur Institute at Naples 810 persons were treated during the years 1886 to 1892, with a mortality of 0.86 per cent.

During the year 1891, 1,564 persons were inoculated at the Pasteur Institute in Paris, with a total mortality of 0.57 per cent. In 324 of these cases the animal which inflicted the bite was proved to be rabid by experimental inoculations.

Horsely (1889) has made a comparison of the results obtained by the "intensive treatment" as compared with those by the treatment first employed, and says:

"It is evident that the intensive treatment is very successful in coping with the worst cases, and that, instead of being itself a source of death, as asserted by those who gain notoriety and subsistence by vilifying and misrepresenting scientific progress, it is a powerful agent in saving life."

The following table is given by Horsely "as showing the contrast between the old or simple treatment and the intensive treatment:"

	Simple Treatment, 1886.	Intensive Treatment, 1888.
Odessa.....	3.39 per cent.	0.64 per cent.
Warsaw	4.1 " "	0.0* " "
Moscow.....	8.2† " "	1.6 " "

Perdrix (1890), in an analysis of the results obtained at the Pasteur Institute in Paris, calls attention to the fact that the mortality among those treated has diminished each year and ascribes this to improvement in the method. He says:

"At the outset it was difficult to know what formula to adopt for the treatment of each particular case. Upon consulting the accounts of the bites in persons who have died of hydrophobia notwithstanding the inoculations, we have arrived at a more precise determination as to the treatment suitable for each case, according to the gravity of the lesions. In the cases with serious wounds we inject larger quantities of the emulsion of cord and repeat

* The figures include sixteen months' work, and thirty individuals bitten in the face—four by wolves.

† This unusually high rate was found to be due to imperfections in the manner of preparing the cords for the inoculation material.

the inoculations with the most virulent material. For the bites upon the head, which are especially dangerous, however slight their apparent gravity may be, the treatment is more rapid, and, above all, more intensive—that is to say, the virulent cord is injected several times."

The statistics arranged with reference to the location of the bite are given by Perdrix as follows :

Bitten upon the head,	684 ;	died, 12,	= 1.75 per cent.
" " " hands,	4,396 ;	" 9,	= 0.2 " "
" " " limbs,	2,839 ;	" 5,	= 0.17 " "

Other methods of making susceptible animals immune against hydrophobia have been proposed and proved by experiment to be successful. Thus Galtier in 1880–1881 claimed that the sheep and the goat could be protected by intravenous injections of the virus of rabies, and more recent experiments fully confirm this. Protopopoff (1888) by injecting an emulsion of cord from a rabid animal into the circulation of dogs succeeded in protecting them from hydrophobia as a result of a subsequent inoculation with virulent material upon the surface of the brain. He injected into a vein, at intervals of three days, 1 c.c. of an emulsion of cord—first of six days, second of three days, third of one day. Roux had previously accomplished the same result by a single intravenous injection of a larger quantity (35 c.c.) of cord which had been kept for five or six days. In discussing his results Roux calls attention to the fact, which had been developed during his experiments, that the virulence of the spinal cord of rabid animals does not depend entirely upon the length of time it has been kept, but that large doses of cord kept as long as twelve days will sometimes produce hydrophobia when injected into the circulation of dogs, when smaller doses of cord kept five or six days prove to be inoffensive.

He supposes that during desiccation the virus may not be equally acted upon throughout the cord, but that certain "islands" in the central portion may remain living and virulent when all the rest has been modified. A practical point with reference to the preservation of virulent material is referred to by Roux in a note published in the "Annales of the Pasteur Institute." This is the fact that when preserved in glycerine portions of the central nervous system retain their virulence for considerable time. Other forms of virus, *e.g.*, vaccine, may also be preserved in the same way.

Centanni (1892) has succeeded in making rabbits immune by inoculating them with an attenuated virus obtained by subjecting virulent material to the action of an artificial gastric juice. After digestion for less than twelve hours the virus still kills rabbits, when inoculated beneath the dura mater, but the period of incubation is considerably prolonged. After from twelve to twenty hours' digestion it no longer kills rabbits, but causes an infection, from which they recover, and after which they are immune.

Serum-therapy.

Tizzoni and Centanni (1892) have reported success in the treatment of infected rabbits by the use of blood-serum from immune animals of the same species—immunized by the "Italian method" above described. The animals experimented upon were inoculated with a "street virus" which produced paralytic rabies in rabbits and caused their death in from fourteen to eighteen days. The blood-serum was obtained from rabbits which had been proved to be immune by resisting inoculations

of virus of full strength on the surface of the brain. The blood-serum, in doses of 3 to 5 c.c., was injected subcutaneously, or into the peritoneal cavity, or into the circulation. Injections were made into each animal (in all from 11 to 26 c.c.) after the first symptoms of paralytic rabies had appeared (on the 7th, 10th, 11th, and 14th day after infection). Four rabbits treated in this way fully recovered. In a subsequent experiment the bacteriologists named treated three rabbits with a dry antitoxin obtained by precipitation from the blood-serum of immune rabbits. The precipitate was obtained by adding 1 part of serum to 10 parts of alcohol, and was dried *in vacuo*. This dried precipitate, in doses of 0.18 to 0.25 gm., was dissolved in sterilized water and injected as in the previous experiment. Commencing on the eighth day after infection five or six doses were given—in all 0.9 to 1.3 gm. All of the animals treated recovered, while all of the control animals died. Babes had previously (1889) reported successful results in conferring immunity upon susceptible animals by injections of blood-serum from immune animals.

Tizzoni and Schwartz, in pursuing this line of investigation (1892), report that while the blood-serum of immune rabbits neutralizes the "fixed virus" of rabies *in vitro*, after short contact (five hours), the blood-serum of immune dogs has but slight antitoxic potency. The immunizing substance in the rabbit serum does not dialyze, is soluble in glycerin, is precipitated by alcohol, and in general behaves like a globulin. In subsequent experiments Tizzoni and Schwartz used blood-serum from dogs and rabbits immunized by Pasteur's method. The blood was drawn from the carotid of the immune animals, and the serum from the same, mixed with virulent spinal mar-

row in the form of a homogeneous emulsion, obtained by crushing and pressing through linen. These experiments corresponded with those previously made as to the superior antitoxic power of rabbit-serum, which, after five hours' contact, neutralized the virulence of the emulsion of cord. By the injection of serum from an immune rabbit, in doses of 5 c.c., into the circulation of other rabbits, they were, as a rule, made immune. The immunizing substance (antitoxin) was shown by other experiments to be present only in the blood. Extracts from the liver, spleen, kidneys, or muscles gave a negative result.

In their latest communication (1894) Tizzoni and Centanni give an account of further experiments made principally upon sheep and dogs. By repeated inoculations they succeeded in obtaining from these animals a serum having an immunizing value of 1 to 25,000 or more, and from this a precipitate was obtained estimated to have a value of 1 to 300,000, and which in doses of 0.23 gm. (of the dried precipitate), dissolved in five times its weight of water, ought to be a sufficient dose to protect a man from the development of hydrophobia after being bitten by a rabid animal.

The authors named believe that inoculations with this antitoxin would be reliable for man, and that they would possess decided advantages over Pasteur's method of inoculation. These advantages are specified as follows:

"Applicability at any time during the period of incubation up to the moment of the appearance of symptoms of rabies; absolute absence of virulence and of any injurious action; very rapid treatment by the injection of one or several small doses of material; complete solubility and consequently prompt absorption of the material injected and its easy preservation in a dry condition."

Finally, the authors say that they are engaged in preparing the antitoxin on a sufficient scale to enable the test to be made upon man, and that for this purpose they are using sheep as the most suitable animals for the purpose.

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X.

INFLUENZA.

The bacillus discovered by Pfeiffer, in 1892, is now well established as the specific cause of this disease. Bruschettini has recently (1893) reported the details of his experiments upon rabbits, for which animals this bacillus is pathogenic. As a result of these experiments he has reached the following conclusions :

"1. Rabbits may be vaccinated against the pathogenic action of cultures of the influenza bacillus without great difficulty.

"2. The best material for producing a high grade of immunity is blood-cultures which have been filtered through the Berkenfeld filter.

"3. The blood-serum of immunized animals has strong antitoxic properties, but has no germicidal power.

"4. The serum of vaccinated animals has the power of conferring immunity upon other animals, in comparatively small amounts—in the proportion of 1 to 42,000 of body-weight, and perhaps still less.

"5. This serum has also a decided curative action, and rescues rabbits from death even as late as forty-eight hours after infection by injection of a culture of the bacillus into the trachea."

These results lead the author to hope that serum-therapy may afford a method of curing this disease in man. For this purpose the blood of an immune rabbit would

appear to be the most promising source from which to procure an antitoxic serum.

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XI.

INFLUENZA IN HORSES.

Schütz (1887) has described a minute oval bacillus, usually associated in pairs, which appears to be the specific infectious agent in the disease known in Germany as *Brustseuche*. This bacillus is pathogenic for mice, rabbits, pigeons, and guinea-pigs, but not for swine or chickens. By injection of cultures into the parenchyma of the lungs Schütz reproduced the disease—confirmed in 1888 by Hell.

Horses which have suffered an attack of infectious influenza are subsequently immune, and the experiments of Hell have shown that an immunity also follows the disease which results from inoculations with pure cultures of the Schütz bacillus.

The extended experiments made by the War Department of the German Government show that the disease is not produced by intravenous injections or by the ingestion of the bacillus with the food. Infection occurs, however, when cultures are injected into the respiratory passages. Subcutaneous injections cause a painful local tumefaction, often followed by an abscess, but without the general symptoms of influenza.

Experiments have been made in Germany, by Hell, Siedamgrotzki, and others, which indicate that the subcutaneous injection of blood-serum from immune horses may confer immunity on other horses. Hell usually in-

jected 40 c.c. at a time, and repeated this at intervals until 200 to 240 c.c. had been injected in the course of two or three weeks. He also reports the results of treatment by injections of blood-serum into the trachea in horses already infected, and thinks these injections had a favorable influence on the course of the disease. Experiments made subsequently by Toepper have given a similar result, but others have not been so fortunate, and the immunizing value of blood-serum injections, as practised by the authors referred to, seems to be still a matter of some doubt. Toepper (1893) gives full directions for collecting the serum and a detailed account of results of experimental inoculations made by himself and others. He prefers to inject the serum into the breast over the ensiform cartilage. No reaction occurs after the injection.

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XII.

PLEURO-PNEUMONIA OF CATTLE.

In a recent communication (1894) to the Central Society of Veterinary Medicine (of France), Arloing claims that he has demonstrated the etiological relation of a bacillus first described by him in 1889 (*Pneumobacillus liquefaciens bovis*) to the infectious disease of cattle known as pleuro-pneumonia. The demonstration was not complete until recently, because of failure to reproduce the disease by inoculation with a pure culture of the bacillus. Arloing asserts that he has now succeeded in accomplishing this, and ascribes his earlier failures to the fact that the bacilli in his cultures were too much attenuated to produce the disease by inoculation. He has found that the bacilli as found in the serum from the lungs become more virulent when they develop in the subcutaneous tissues of an animal; and cultures made from the bacillus procured from the subcutaneous tissues at the point where protective inoculations are usually practised—the tail—proved to be sufficiently active to reproduce the disease when they were injected into the lungs. Chauveau, who was present at the meeting when Arloing read his most recent paper on the subject (June 28, 1894), states that he has seen the inoculated animals and has no doubt that the bacillus in question is the infectious agent.

Although this demonstration is of such recent date, protective inoculations against this disease have long

been successfully practised. For this purpose serum obtained from the lungs of an animal recently dead has been employed, this having been proved by experiment to be infectious material although the exact nature of the infectious agent present in it was not determined.

Willems, who was one of the first to advocate the use of protective inoculations in this disease (1852), has recently given a lecture (1894) in which he has reviewed the evidence in favor of these inoculations in the disease under consideration. Various methods have been employed. Thus Willems states that the natives of the banks of the Zambéze cause animals to swallow a certain quantity of the liquid from the pleural cavity of an animal recently dead, and thus give them immunity. The virus has been injected into the circulation by some experimenters, and others have proposed to attenuate it by heat. But the method which has been most extensively employed is that discovered by the Dutch settlers at the Cape of Good Hope (the Boers), and consists in inoculating animals in the tail with serum from the lungs of an animal recently dead; or with a virus obtained from the tumefaction produced by such an inoculation in the tail. This secondary virus was very extensively used by Lenglen, a veterinarian at Arras, who communicated his results to the Academy of Science at Paris, in April, 1863, and Willems says, in his last published communication, that this is the method which he prefers. It is also the method most extensively employed in Australia, into which country infectious pleuro-pneumonia was introduced in 1858. It quickly spread and has caused enormous losses. The killing of all animals, sick or suspected of being infected, was tried for several years; but this proved to be ineffectual for stamping out the disease, and the sacrifice

was so great that this measure of prophylaxis was abandoned.

According to Loir, attention, in Australia, was called to Willem's method of protective inoculations, in 1861, by a letter from Cape Colony published in the journals of Sydney and in Melbourne. The method was at once applied both in Victoria and in New South Wales, and since that date many thousands of cattle have been inoculated. In order to obtain a sufficient supply of virus the method recommended by Pasteur in 1882 has been followed. This is described by Pasteur himself in the following words :

"With a single lung we may procure sufficient virus to serve for numerous series of animals. And without having recourse to other lungs this provision may be maintained in the following manner : It is sufficient before the supply of virus is exhausted to inoculate a young calf in the dewlap or in the shoulder. The animal dies very promptly, and all its tissues near the point of inoculation are infiltrated with serum, which is virulent, and may be collected and preserved in a state of purity."

Loir prefers to obtain the virus in this way from a calf six to twelve months old, during the second week after inoculation, when the temperature of the animal has gone up to 40° to 42° C., as the virus is then said to possess the maximum degree of intensity. This vaccine seems to become attenuated in passing through a series of animals by inoculation, so that when it has been passed through a series of five animals it no longer produces death even when inoculated in the most dangerous localities. Loir testifies to the protective value of inoculations with this virus made in the tail of the animal, and gives the following example : A few months prior to the publication of his paper (1893), about two thousand cows were inocu-

lated with a virus which had been passed through a series of five calves. At the moment of being driven away they were joined by nineteen other cows not vaccinated. After being on the road for a distance of two thousand kilometres the animals arrived at their destination. The two thousand vaccinated were in good condition, while eight of the non-vaccinated had died of pleuro-pneumonia.

In the *Bulletin of the Central Society of Veterinary Medicine* of May 24, 1894, M. Robcis reports the results of inoculations made with cultures of Arloing's *Pneumobacillus liquefaciens bovis*, and with injections of pulmonary serum. His statistics with reference to the last-mentioned "legal" inoculations he has obtained from official documents relating to the Department of the Seine.

The total number of infected localities in this department during the years 1885 to 1891 was 1,253; total number of contaminated animals, 18,356; total number inoculated, 18,359; total number of deaths prior to inoculation, 1,753; total number of deaths after inoculation, 2,741; total number of deaths due to the inoculation, 94; total percentage of mortality, 22.8 per cent. After discussing these and other statistics Robcis arrives at the conclusion that Arloing's method of preventive inoculations with cultures of the *Pneumobacillus liquefaciens bovis* gives better results than the legal method with serum from an infected animal, the total loss among animals exposed to contagion not being over twelve to fourteen per cent.

Nocard (1892) says that serum from the lungs of an animal dead from pleuro-pneumonia preserves its virulence and usefulness as a vaccine, when mixed with half a volume of pure neutral glycerin and half a volume of a five per cent. solution of carbolic acid. At the end of two and a half months this mixture preserved its full virulence.

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XIII.

PNEUMONIA.

The micrococcus of croupus pneumonia was discovered by the present writer in the blood of rabbits inoculated subcutaneously with his own saliva in September, 1880. In 1885 this micrococcus, which I had repeatedly obtained in pure cultures from the blood of rabbits inoculated, as in the first instance, with my own saliva, was identified with the micrococcus of the same form present in the rusty sputum of patients with pneumonia. In a paper read before the Pathological Society of Philadelphia, in April, 1885, and published in the *American Journal of the Medical Sciences* on July 1st of the same year, I say :

"It seems probable that this micrococcus is concerned in the etiology of croupous pneumonia, and that the infectious nature of the disease is due to its presence in the fibrinous exudate into the pulmonary alveoli."

This has since been fully established by the researches of Fränkel, Weichselbaum, Netter, Gameleïa, and many others. Fränkel first discovered this micrococcus in his own salivary secretions in 1883, and his first paper relating to its presence in the exudate of croupous pneumonia was published on July 13, 1885, *i.e.*, thirteen days after the publication of the paper from which the above quotation is made. Under these circumstances the

writer feels justified in again calling attention to his priority in the discovery of this important pathogenic micrococcus, and in objecting to its being described as "Fränkel's pneumococcus," the "diplococcus of Fränkel," etc.

In my paper above referred to (July, 1885) I described this micrococcus under the name of Micrococcus Pasteuri, but in my "Manual of Bacteriology" (1892) it is described under the name of *Micrococcus pneumoniæ crouposæ.*

This micrococcus is very pathogenic for mice and for rabbits, less so for guinea-pigs and for dogs. Like other pathogenic microörganisms of the same class it varies greatly in virulence when obtained from different sources. In the saliva of healthy persons, which seems to be its normal habitat, it sometimes has comparatively little virulence. On the other hand, when contained in the blood or in an exudate from a serous cavity of an infected rabbit or mouse, it is very virulent. In one instance (1881) the writer has seen a fatal result in a dog from the subcutaneous injection of 1 c.c. of bloody serum from the subcutaneous connective tissue of a rabbit recently dead.

Pneumonia never results from subcutaneous injections into susceptible animals, but injections through the thoracic walls into the lung may induce a typical fibrinous pneumonia. This was first demonstrated by Talamon (1883), who injected the fibrinous exudate of croupous pneumonia, obtained after death, or drawn during life by means of a Pravaz syringe, from the hepatized portion of the lung, into the lungs of rabbits. Gameleïa has also induced pneumonia in a large number of rabbits, and also in dogs and sheep, by injections directly into the pulmonary tissue. Sheep were found to survive

subcutaneous inoculations, unless very large doses (5 c.c.) of a virulent culture were injected. But intrapulmonary inoculations are said to have invariably produced a typical fibrinous pneumonia which usually proved fatal. In dogs similar injections gave rise to a "frank, fibrinous pneumonia which rarely proved fatal, recovery usually occurring in from ten to fifteen days, after the animal had passed through the stages of red and gray hepatization characteristic of this affection in man."

Without doubt an attack of pneumonia is followed by a certain degree of immunity of longer or shorter duration. According to Ruge, who has recently made a careful study of the subject, relapses are very infrequent—indicating a temporary immunity—but subsequent attacks are more likely to occur in those who have once suffered an attack of the disease, and as many as four or five attacks have been known to occur in the same individual.

In 1,100 cases collected by Wagner but two relapses occurred (= 0.18 per cent.). Ruge reports that in 440 cases treated at the Charité in Berlin there were but two relapses. The liability to subsequent attacks at a later period is shown by the following figures, which we copy from Ruge's paper: In 280 cases reported by Stortz, 26.4 per cent. had previously suffered an attack of the disease; in 133 cases reported by Morhart the proportion of previous attacks was 41.3 per cent.; in 157 cases by Pohlmann, 34.4 per cent.; in 166 cases by Schapira, 31.3 per cent.; in 128 cases by Keller, 36.9 per cent.; in 175 cases by Grisolle, 30.9 per cent.

The writer, in a series of experiments made during the winter of 1880–81, obtained experimental evidence which showed that susceptible animals (rabbits) acquire immunity from the pathogenic action of this micrococcus

as a result of inoculations with an attenuated virus. The experiments referred to had as their object the determination of the comparative value of various germicidal agents, as tested upon this micrococcus; incidentally it was found "that a protective influence has been shown to result from the injection" (into rabbits) "of virus, the virulence of which has been modified, without being entirely destroyed, by the agent used as a disinfectant." (Quoted from the writer's report of the experiments referred to, Johns Hopkins University, "Studies from Biological Laboratory," Baltimore, 1882.)

In 1891 G. and F. Klemperer published an important memoir relating to the pathogenic action of this micrococcus and the production of immunity in susceptible animals by means of filtered cultures. In some cases this immunity was found to last as long as six months. A curious fact developed in their researches was that the potency of the substance contained in the filtered cultures was increased by subjecting these to a temperature of 41° to 42° C. for three or four days, or to a higher temperature (60° C.) for an hour or two. When injected into a vein after being subjected to such a temperature, immunity was complete at the end of three or four days; but the same material, not so heated, required larger doses and a considerably longer time (fourteen days) to confer immunity upon a susceptible animal. The unwarmed material caused a considerable elevation of temperature, lasting for some days. The authors mentioned conclude from their investigations that the toxic substance present in cultures of Micrococcus pneumoniæ crouposæ is a proteid substance, which they propose to call pneumotoxin. The substance produced in the body of an immune animal, as a result of protective inocula-

tions, upon which the immunity of these animals depends, is also a proteid, which they call antipneumotoxin. This they isolated from the blood-serum of immune animals. By experiment they were able to demonstrate that the blood-serum containing this protective proteid, when injected into other animals, rendered them immune; and also that it arrested the progress of the infectious malady induced by inoculating susceptible animals with virulent cultures of the micrococcus. When injected into the circulation of an infected animal its curative action was manifested by a considerable reduction of the body temperature. The toxalbumin was obtained from filtered bouillon cultures of a virulent variety of the micrococcus of pneumonia, in the form of an amorphous yellowish-white powder. This was thrown down from the filtered cultures by means of alcohol, and again dissolved in water and reprecipitated in order to purify it.

Issaeff (1893) as a result of his experiments has found that the virulence of this micrococcus can be greatly increased by successive inoculations in the peritoneal cavity of rabbits, and that after a series of ten or twelve such inoculations the blood of the infected animal does not coagulate and becomes extremely toxic. In order to obtain the toxins from this blood Issaeff collects the blood of three or four animals just dead in a sterilized vessel, and adds to this an equal volume of sterilized water containing one per cent. of glycerin, made alkaline by the addition of a few drops of a concentrated solution of bicarbonate of soda. The mixture is sterilized by passing it through a Chamberland filter. This liquid sometimes kills rabbits when injected into the circulation in the proportion of one per cent. of the weight of the

animal. When heated to 70° C. its toxic power is considerably diminished and a temperature of 100° C. neutralizes it completely.

Emmerich (1891) has succeeded in immunizing rabbits and mice by the intravenous injection of a very much diluted but virulent culture of the micrococcus. Other rabbits and mice were rendered immune by injecting into them material obtained from rabbits immunized with diluted cultures. The flesh of these animals was rubbed up into a pulp, and the juices obtained by pressure through a piece of sterilized cloth. The bloody juice, after standing for twelve hours at a temperature of 10° C., was passed through a Pasteur filter and then served to immunize the animals referred to.

Belfanti (1892) has succeeded in immunizing rabbits against the pathogenic action of this micrococcus by injecting into the circulation a filtrate obtained from the sputa of pneumonia cases. The viscid sputa mixed with an equal part of distilled water was kept on ice for twenty-four hours and then passed through a Chamberland filter. Ten c.c. of this filtrate was injected into the ear vein of rabbits. Some of the animals so treated proved to be immune against general infection when inoculated with a virulent culture of the micrococcus, but they had a localized inflammation and œdema about the point of inoculation. After recovering from this they proved to be entirely refractory against subsequent inoculations.

Foà and Scabia (1892) have reported success in producing immunity with filtered cultures, and also with a glycerine extract from the blood of an infected rabbit. This, after filtration, was injected subcutaneously in doses of 2 c.c. at intervals of five days. The authors named

have also produced immunity in rabbits by the use of "pneumo-protein." This is an extract from the bacterial cells obtained by first collecting these from the surface of a Chamberland filter through which the cultures have been passed; then digesting them for three hours at 55° C. in a five per cent. solution of glycerin. According to Foa and Scabia immunity produced in this way is more decided and of longer duration than that resulting from the other methods tested by them.

Mosny (1892) has also made numerous experiments which show that rabbits may be immunized by means of filtered cultures, or by the juices from the tissues of an immune animal obtained by maceration and filtration. When sterilized cultures were employed the best results were obtained by first heating very virulent cultures for three hours at 60° C. The dose employed was 10 c.c., and immunity was not established immediately but required a period of at least four days for its development.

The blood-serum of immune rabbits was not found to have any bactericidal power, and the micrococcus of pneumonia preserved its vitality longer in the blood-serum of immune rabbits than in that of other animals of the same species.

G. and F. Klemperer had previously reported that the blood of immune rabbits does not destroy the micrococcus of pneumonia or restrict its development.

Issaeff (1893) also reports his success in immunizing rabbits by means of sterilized cultures or filtered blood from infected animals recently dead. A single intravenous injection of 10 c.c. of filtered blood, prepared as heretofore indicated (p. 220) sufficed to confer immunity. To test immunity the animals were subsequently inoculated with two to four drops of virulent blood; and to maintain

it the inoculations (0.5 c.c.) were repeated every four weeks. Although immune against infection these animals are said not to have acquired any immunity against the toxins of the micrococcus of pneumonia. Contrary to the conclusion reached by G. and F. Klemperer, Issaeff concludes from his experiments that "rabbits, although completely refractory against pneumonic infection, remain highly sensitive to the toxins of this microbe. Even small doses of the toxins are not neutralized in the blood of vaccinated animals. We are therefore brought to the conclusion that the existence of an antitoxic property of the blood of vaccinated animals cannot be admitted."

The serum of immunized rabbits was not found by Issaeff to possess any bactericidal power for the micrococcus of pneumonia, and no attenuation of virulence occurred as a result of cultivation in this serum. But when introduced beneath the skin of an immune rabbit the micrococcus quickly loses its virulence. At the end of eighteen hours it has completely lost its pathogenic power, and cultures made in bouillon no longer have any injurious effect upon rabbits. This attenuating effect produced in the body of an immune animal is ascribed by Issaeff to the action of phagocytes, which are said to be very numerous, and in the course of five or six hours to pick up all of the cocci in the vicinity of the point of inoculation. These are not, however, immediately destroyed in the interior of the phagocytes, but preserve their vitality for nearly forty-eight hours, and when introduced into bouillon give a culture which has no longer any pathogenic virulence.

Serum-therapy.

G. and F. Klemperer (1891) having succeeded in immunizing rabbits against infection by this micrococcus proceeded to experiment with the blood-serum of immune rabbits as a therapeutic agent. They were successful in saving infected rabbits by the intravenous injection of such blood-serum in the dose of 8 c.c. administered as late as twenty-four hours after the subcutaneous injection of a virulent culture. Subcutaneous injections were less effective, and often failed after an interval of twenty-four hours from the time of infection, but were successful when given within eight or ten hours. The tissue juices of immune rabbits also proved to have a curative power.

They then proceeded to test the therapeutic value of immune rabbit's serum on man by injecting, in six cases, from 4 to 6 c.c. beneath the skin. They report that in every case a notable fall of temperature followed the injection. In two cases it remained normal, and in two again mounted to the former point at the end of six hours. The authors referred to recognized the fact that further experiments would be required to establish the value of the treatment.

Mosny (1892) was not able to confirm the results of G. and F. Klemperer as to the therapeutic value of blood-serum or tissue juices of immune animals when injected into rabbits after infection, even when this was done after a very short interval.

On the other hand, Foà and Scabia (1892) succeeded in curing infected mice and rabbits by the subcutaneous injection of blood-serum from immune animals. But in ten cases of pneumonia in man, treated with doses of 5 to 7

c.c. of blood-serum from immune rabbits, their results were less favorable than those reported by the Klemperers. The dose mentioned was repeated two or three times at intervals of two days. In six of the ten cases no modification of the course of the disease occurred, and the crisis took place on the ninth or tenth day. In the other four cases the crisis occurred in from twenty-four to forty-eight hours after the first injection, but it would of course not be safe to conclude that this was a result of the dose given.

Emmerich (1894), referring to the experiments made by Klemperer and by Foà, says that the rabbits from which they obtained their serum were not completely immunized. In his own experiments such a degree of immunity was acquired that the animals resisted doses of 30 to 40 c.c. of a virulent culture. This immunity did not, according to Emmerich, depend upon the presence of an antitoxin in the blood of the immune animal, but upon the destruction of the micrococci when introduced into the body of the animal by some bactericidal substance present in the blood. Emmerich and Fowitzky have given the results of their experiments in serum-therapy in a paper published in 1891. These were not numerous, but indicated that the blood-serum of rabbits immunized by their method has the power of curing the acute infectious disease (septicæmia) produced in susceptible animals (rabbits and mice) by inoculation with a culture of the micrococcus of croupous pneumonia. In Emmerich's last published paper upon the subject he gives no additional experimental evidence but says :

"That the active substance (*Heilstoffe*) in the serum of immune animals will also some time prove to have an

ideal curative power for man is beyond doubt and only a question of time."

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XIV.

RINDERPEST.

The disease of cattle known in Germany as rinderpest is due to a bacillus closely resembling the bacillus of fowl cholera and of swine plague (*Bacillus septicæmiæ hemorrhagicæ*).

Professor Semmer, of St. Petersburg, has reported (1892) his success in immunizing cattle against this disease. The virulence of cultures was attenuated by passing them through guinea-pigs, or by exposure to heat, and this attenuated virus was used in protective inoculations. Semmer says:

"By the subcutaneous injection of blood-serum from immune animals their susceptibility to rinderpest was diminished, and such blood-serum destroyed the 'rinderpest contagium' in one to twenty-four hours."

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XV.

SMALL-POX.

Inoculations with virus obtained from a pustule on a small-pox patient were extensively practised before the discovery of vaccination by Jenner. These inoculations gave rise to a mild attack of the disease, followed by immunity which was apparently as complete as that following a more severe attack contracted in the usual way. This method seems to have been practised by Eastern nations long before it was introduced into Europe. It was extensively employed in Turkey early in the eighteenth century, and was introduced into England through the influence of Lady Mary Wortley Montagu. No doubt the mortality from small-pox was greatly diminished by these inoculations, but they were attended by the disadvantage that the disease was propagated by them, inasmuch as inoculated individuals became a source of infection for others. Inoculation was still practised in England for some time after the demonstration of the protective value of vaccination, but in 1840 it was prohibited by an act of Parliament. The usual course of the disease as a result of inoculation was as follows: On the second day a papule developed at the point of inoculation, which became vesicular on the fourth day, and pustular on the eighth, at which time the patient had a chill, followed by fever and tumefaction of the axillary

glands. This was followed by the ordinary small-pox eruption as seen in a mild case.

There is some evidence that vaccination as a protection against small-pox was practised to a limited extent prior to the time of Jenner. Thus Von Humboldt has stated that it was known at an early period to the Mexicans. But its introduction as a reliable method of protecting against small-pox is due to the patient researches of the renowned English physician, whose attention was first attracted to the subject in 1768, although it was not until 1796 that he made his first vaccination in the human subject. His first public institution for the practice of vaccination was established in 1799, and the following year the practice was introduced into France, Germany, and the United States.

Vaccinia in the cow was more frequent before the introduction of vaccination than at present, and often prevailed as a veritable epidemic. This was no doubt due to the greater prevalence of small-pox, and also to the fact that milkers, not protected by vaccination, by the sores on their hands, arising from contact with the teats of an infected cow, communicated the disease to other animals.

We cannot attempt to review the evidence for and against the view that vaccinia in the cow is simply a modified form of small-pox, rather than a specific infectious disease of the bovine species, as has been claimed by numerous physicians of prominence. We believe, however, that the former view is supported by experimental evidence, as well as by the analogy resulting from recent additions to our knowledge relating to protective inoculations in other infectious diseases.

Nor is it necessary to devote any space to the methods

of propagating vaccine virus, and the operation of vaccinating, with its immediate results. But some account of the value and duration of the immunity conferred by a successful vaccination seems desirable.

In the early days of vaccination it was claimed that a successful vaccination would confer a life-long immunity against small-pox; but this has been disapproved by ample experience, and it is now generally recognized that the immunity usually expires after a time, which varies greatly in different individuals, and can only be determined for each by a revaccination practised at proper intervals, or when small-pox is prevalent. The fact that a single attack of small-pox is not always protective would lead us to expect that the immunity from vaccination would not be absolute, and experience shows that in every small-pox epidemic a certain number of persons who have been vaccinated fall victims to the disease; but experience also shows that the mortality among the vaccinated is very much less than among the non-vaccinated. The London Board of Health, from thirty returns sent to them—not selected—has given the following table:

	Number of Cases.	Deaths.	Percentage of Deaths.
Natural small-pox in the unprotected	1,731	361	20.85
Small-pox after small-pox	58	22	37.92
Small-pox after vaccination	929	32	3.44

The gradual loss of immunity from vaccination, as determined by revaccination, is shown by the following figures, which are given in a recent (1894) paper by Bie-

dert. In the year 1889 small-pox was introduced into Hagenau, and twenty-one cases occurred. The physician officially charged with that duty revaccinated all of the school-children with the result given below:

Age.	Number Vaccinated.	Successful.	Per Cent.
6 to 7.....	288	94	33.0
7 to 8.....	292	187	63.8
8 to 9.....	222	161	72.5
9 to 10.....	221	176	80.0
10 to 11.....	306	273	85.8
11 to 12.....	413	367	88.6

We may remark in connection with this table that in Germany vaccination is compulsory, and all children must be vaccinated before the September of the year following their birth. All scholars in public or private schools who have not had an attack of small-pox must be revaccinated when twelve years old.

The experimental evidence presented in the section on acquired immunity (Part First, Section II.) suggests the possibility that in small-pox and other infectious diseases, in which the specific infectious agent has not yet been demonstrated, the immunity resulting from an attack—or in small-pox from vaccination—may also be due to the presence of an antitoxin in the blood of the immune individual.

The writer undertook some experiments with a view to determine this question in the spring of 1892, but owing to various causes has never been able to complete the investigation. The experiments made, however, indicate that the blood of a recently vaccinated calf contains an antitoxin which neutralizes the potency of vaccine virus, either bovine or humanized. The experiments are re-

corded in my paper on the "Practical Results of Bacteriological Researches" as follows:

These experiments were made with the kind assistance of Dr. Wm. E. Griffiths, of Brooklyn, who has for many years been engaged in the production of vaccine virus, and consequently is an expert in the vaccination of calves and in recognizing vaccinia in these animals.

"Upon visiting Dr. Griffiths and making known to him my desires, I found him quite willing to assist me, and also that he had a recently vaccinated, and consequently immune, calf in his stable. This animal had been vaccinated in numerous places upon the abdomen and thighs fourteen days previously. The vaccination was entirely successful, and a large number of quills had been charged from the vesicles which formed. At the time of my visit for the purpose of collecting blood-serum from this animal, dry crusts still remained attached at the points where vaccination had been practised two weeks previously. On the 28th of April I collected blood-serum from a superficial vein in the hind leg of this calf. This blood was placed in an ice-chest for twenty-four hours, at the end of which time the clear serum was drawn off in 'Sternberg's bulbs.' Four drops of this serum were placed in each of two small, sterilized, glass tubes; in one of these we placed three quills charged with fresh vaccine lymph from a calf. At the end of an hour the quills were removed, after carefully washing off in the serum the lymph with which they had been charged. In the other tube we mixed with the four drops of blood-serum an emulsion made from a fragment of a perfectly fresh vaccine crust from the arm of a child; this was crushed upon a piece of glass and rubbed up with a little of the same blood-serum. The two tubes were now placed in an ice-chest for twenty-four hours, at the end of which time the contents were used to vaccinate a calf purchased for the purpose. Dr. Griffiths carefully shaved the thighs of this calf and scarified each thigh in several

places, as he is accustomed to do in vaccinating for the propagation of lymph. The contents of the tube containing lymph from the quills was rubbed into the scarified places upon one thigh, and the contents of the tube containing the emulsified crust into the other. On the 8th of May, nine days after the vaccination, the calf was carefully examined, and it was ascertained that the result of the vaccination was entirely negative.

"Evidently it was necessary to make a control experiment before we would be justified in ascribing this negative result to a neutralization of the virus by some special substance present in the blood-serum of an immune calf. Possibly the blood of a non-immune calf might also, after an exposure of twenty-four hours, neutralize the specific virulence of vaccine lymph. The control experiment was made as follows:

"On the 9th day of May we collected blood from a vein in the leg of a non-immune (not vaccinated) calf; this was placed in the ice-chest for twenty-four hours, and the following day clear serum was collected in Sternberg's bulbs. Three quills, charged with fresh lymph from a calf, of the same lot as those used in the previous experiment, were placed in four drops of this blood-serum in each of two small glass tubes. As in the previous experiment, the lymph was washed from the quills at the end of an hour, and the tubes were placed aside in the ice-chest. At the end of twenty-four hours the serum in these two tubes was used to vaccinate the same calf which had served for the previous experiment. Several points were scarified upon the left thigh and upon the left side of the abdomen, which were carefully shaved for the purpose.

"At the same time the animal was vaccinated upon the right thigh and upon the right side of the abdomen with virus mixed with the blood-serum from the immune calf. This serum, collected in Sternberg's bulbs on the 28th of April, had since been kept in the ice-chest. One hour before the vaccination four drops of this blood-serum were

mixed with one drop of liquid lymph, which had been recently collected by Dr. Griffiths in a capillary tube from a vaccinated calf. At the same time three quills charged with bovine lymph were immersed in four drops of the same blood-serum—from immune calf. As stated, the animal was vaccinated upon the right side of the abdomen and upon the right thigh with this virus, which had been exposed for one hour to the action of blood-serum from an immune calf. The serum containing the liquid lymph was rubbed into the scarification on the right side of the abdomen, the serum containing lymph from the quills into the right thigh. On the 19th of May, eight days after the vaccination, the animal was carefully examined by Dr. Griffiths and myself, and the following results noted: Upon the left thigh and left side of the abdomen the vaccinations—from quills in non-immune blood-serum after twenty-four hours' contact—were entirely successful, the scarification being surrounded by characteristic vesicles and covered by characteristic crusts. Upon the right thigh—vaccinations from quills immersed in blood-serum from immune calf for one hour—and upon the right side of abdomen—vaccinations with liquid lymph mixed with blood-serum from immune calf—the result was entirely negative. Several of the scarifications had entirely healed; others were covered with a dry scab which was easily detached and under which the scarification was healing without any appearance of vesicles such as surrounded the scarifications upon the left side." *

Later I made a number of experiments upon unvaccinated children in two orphan asylums in Brooklyn, with a view to ascertain whether blood-serum from an immune calf, or from an individual who had recently suffered an attack of small-pox, if injected into the subcutaneous

* These results have since been confirmed by Kinyon.

tissues at the time of vaccination, would prevent the development of a characteristic vaccine vesicle. In these experiments from 1 to 5 c.c. of the serum supposed to contain an antitoxin of small-pox was injected near the point of vaccination, or, in some instances, into the other arm. The result was negative, even when serum was used from an individual who was just convalescent from a severe attack of small-pox. But it may be that a different result would have been obtained if a larger quantity of blood-serum had been used, or if it had been injected into the circulation instead of into the subcutaneous tissues. More recent experiments by Kramer and Boyce (1893) and by Landmann (1894) also show that small amounts of serum from immune calves (5 to 10 c.c.) do not prevent the development of the vaccine vesicle; and that blood-serum (25 c.c.) from one who had suffered a recent attack of small-pox did not have any noticeable effect upon the development of a confluent case of small-pox in a child five years of age (Landmann).

This line of research, which appears to us well worthy of attention, is still open for investigators. Immune calves may be obtained at any vaccine establishment, and it is a simple matter to collect sterile blood-serum in considerable amounts from these immune animals. We have been anxious to see a test made as regards the possible therapeutic value of such serum. It could be injected into the circulation of a small-pox patient through the median vein, and there is no reason to suppose that there would be any danger in introducing a considerable quantity in this way. As the writer is not likely to have an opportunity to follow up these experiments he takes this occasion for inviting the attention of others, more favorably

situated for such an investigation, to this promising field for experimental work.*

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* Since the above was written, Kinyon has reported two cases in which blood-serum from an immune calf was injected subcutaneously in small-pox patients in doses of 15 to 30 c.c. with supposed good results in one.

XVI.

SWINE PLAGUE.

As stated in the chapter on cholera in fowls, the bacillus of swine plague (*Schweineseuche*, Löffler and Schütz) very closely resembles Pasteur's microbe of fowl cholera and Koch's bacillus of rabbit septicæmia, and if not identical with these at least varies from them so slightly in its morphological and biological characters that recent authors do not feel justified in considering it a distinct species. Koch first obtained his bacillus of rabbit septicæmia by inoculating rabbits with putrefying flesh infusion. Gaffky produced the same infectious disease in rabbits by inoculating them with impure river-water. Davaine had previously obtained similar results by inoculating rabbits with putrefying blood. The writer in 1887 produced the same disease in rabbits, while in Cuba, by inoculating them with putrefying liver from a yellow fever cadaver. A similar, and possibly identical, bacillus has been found in the blood of deer (Hueppe), of cattle (Kitt), and of buffalo (Oreste-Armanni) suffering from a fatal infectious disease. And all of these allied species or varieties are included by Hueppe and by the present writer under the single specific name *Bacillus septicæmiæ hemorrhagicæ*. The bacillus of the disease known in this country as swine plague, according to Smith, agrees in all particulars with that of the German swine plague (*Schweineseuche*) described by Löffler and

Schütz, except that the latter is more pathogenic for swine and for rabbits.

In a recent publication Smith and Moore (United States Department of Agriculture, Bureau of Animal Industry, Bulletin No. 6, 1894) have given an account of their experiments relating to immunizing animals against the pathogenic action of this bacillus. The bacilli used in these experiments were sufficiently virulent to kill rabbits in twenty hours when injected beneath the skin of these animals in doses of 0.001 c.c. of a fresh bouillon culture. The experiments were made upon young adult rabbits by various methods, viz.: with sterilized bouillon cultures; with sterilized suspensions of agar cultures; with the filtrate of agar suspensions; with defibrinated, sterilized blood of infected rabbits; with blood-serum from immune animals.

"A greater or less degree of immunity was produced in rabbits by sterilized bouillon cultures, sterilized agar suspensions, sterilized blood from infected rabbits, and blood-serum from immunized rabbits. The sterilized blood of diseased rabbits was capable of producing immunity, while the blood-serum of immune rabbits produced rather equivocal results."

The different degrees of immunity which may be acquired by rabbits, as shown by a subsequent inoculation with virulent material, are classified by Moore as follows:

1. No resistance—acute septicæmia.
2. Slight resistance—peritonitis.
3. Increased resistance—pleuritis and pericarditis with or without secondary pneumonia.
4. Higher degree of resistance—pleuritis and peritonitis.

5. Still greater resistance — irregular lesions in the form of abscesses, subcutaneous and subperitoneal.

6. Nearly complete immunity—very slight reaction at the point of inoculation.

Up to the present time the bacteriological experts of the Department of Agriculture have not proposed to make a practical application of the facts developed in their experimental work in the way of protecting herds of swine by means of inoculations with an attenuated virus, or with sterilized cultures. In the report on swine plague, made by the Bureau of Animal Industry, published in 1891, the following measures for arresting an epidemic are recommended:

"When the disease has actually appeared in a herd the question generally arises whether it is worth while to make any attempt to save a portion of the herd or to leave them to their fate. As a rule it may be stated that it is best to slaughter both healthy and diseased at once, and give the surroundings sufficient time to rid themselves of the infection before fresh animals are brought into them. If this be not desirable, we should recommend the following measures to be rigorously carried out:

"*a*. Removal of still healthy animals to uninfected grounds or pens as soon as possible.

"*b*. Destruction of all diseased animals.

"*c*. Careful burial or burning of carcases.

"*d*. Repeated thorough disinfection of the infected premises.

"*e*. Great cleanliness both as to surroundings and as regards food."

In the same report (1891) the following reference is made to protective inoculations:

"As regards swine plague the experiments which have thus far been carried out indicate that this disease may

prove amenable to preventive inoculation. We have been able, by the injection of both living cultures and those sterilized at a low temperature (58° C.) to make the most susceptible animals—rabbits—insusceptible to the most virulent swine-plague bacteria. By two subcutaneous injections of cultures of swine-plague bacteria, swine have been made insusceptible to doses injected into the circulation which proved fatal to control pigs within twenty-four hours."

According to Smith the experiments of Metchnikoff (1892), reported as made with the bacillus of hog cholera, were in fact made with the bacillus of swine plague; we therefore refer to them here. These experiments showed that rabbits could be easily immunized against the pathogenic action of virulent cultures by means of blood, from an infected animal, sterilized by heat. Doses of 1.5 c.c., or more, were fatal to rabbits; but smaller doses, repeated several times, given either subcutaneously or by injection into the circulation, caused the animal to become immune.

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XVII.

STREPTOCOCCUS INFECTION.

It is now generally recognized by pathologists that erysipelas, puerperal fever, certain forms of diphtheritic inflammation of the fauces, and certain acute abscesses are due to infection by a streptoccus described by recent authors under the name of *Streptococcus pyogenes*. This streptococcus, like other pathogenic microörganisms of the same class, varies greatly in its pathogenic power as a result of conditions relating to the source of the particular variety under cultivation. As obtained from a case of erysipelas or puerperal fever it is extremely virulent, but when it has led a saprophytic existence for some time, or has been cultivated for a considerable time in the usual artificial culture media, its pathogenic potency is greatly diminished.

Mironoff (1893) has made a series of experiments with a view to determining whether rabbits can be immunized against the pathogenic action of this streptococcus, and has obtained successful results by the following method:

Vigorous rabbits, weighing 2 kilogm., were inoculated subcutaneously with from 3 to 6 c.c. of a sterilized bouillon culture of the streptococcus. Cultures three days old were employed, and these were sterilized for twenty minutes at 120° C.—the reason for using so high a tem-

perature is not apparent, inasmuch as this streptococcus is destroyed in a few minutes by a temperature of 60° C. At the end of ten to fifteen days "when the animal has fully recovered," a second dose of from 6 to 12 c.c. of a culture, sterilized in the same way, is injected beneath the skin. After another interval of ten to fifteen days 2 c.c. of a virulent non-sterilized culture is injected subcutaneously, and this is repeated with gradually increasing doses (1 to 2 c.c. more) at intervals of the same period. Finally the animals "support without reaction" a dose five times as great as would be required to kill an animal of the same weight not immunized. But the author adds that more than half the animals thus treated died before the completion of the immunizing process. These deaths resulted from local infectious processes, such as peritonitis, pericarditis, meningitis, or abscesses formed at the point of inoculation.

Further experiments showed that the blood-serum of animals immunized in this way when injected into susceptible animals (rabbits) in the dose of 1.5 c.c. per kilogm. of body-weight conferred upon them a certain degree of immunity against streptococcus infection, and with twice this amount (3 c.c.) a very decided immunity was produced. The blood-serum of immune rabbits in doses of 3 to 4 c.c. per kilogm. of body-weight was found to exercise a curative power, and to completely arrest the acute septicæmia resulting from inoculations with a virulent culture of this streptococcus, or to cause the disease to run a chronic course, with formation of abscesses and final recovery.

In this connection we may call attention to the experiments of Emmerich (1886), which show that the fatal course of anthrax infection, in rabbits, may be arrested by

the subcutaneous or intravenous injection of this streptococcus. Subsequent experiments by Emmerich and de Mattei (1887) showed that eleven hours after such an injection the anthrax bacilli were all dead and were already undergoing degenerative changes.

In a recent communication (1894) Emmerich and his associates have reported numerous additional experiments which show that the blood-serum of a rabbit which is suffering from streptococcus septicæmia (third day), when filtered through a Pasteur-Chamberland filter to remove all living cocci, may be used with success in arresting anthrax infection in rabbits. The filtered serum was given four hours after the anthrax infection in the dose of 25 c.c. in the peritoneal cavity and 15 c.c. subcutaneously. This was repeated the following day at nine o'clock in the morning and five o'clock in the evening, and again on the third day in the morning. Favorable results were also obtained by using in the same way blood-serum from a sheep infected with the streptococcus.

Cobbett (1894) reports success in immunizing rabbits by means of attenuated varieties of the streptococcus or by filtered cultures. Also that cutaneous erysipelas, produced by inoculation, after recovery leaves the patient immune from a repetition of the local inflammatory process as a result of a subsequent inoculation, and also confers a general immunity against streptococcus infection. But this immunity is of short duration, not lasting longer than a few weeks. Inoculation in the ear of a rabbit, protected by a previous inoculation in the same locality, is followed by an inflammatory reaction; but this is of brief duration and has disappeared before the erysipelatous inflammation produced in a control is well under way.

We may also mention here the experiments of Mircoli (1894) with reference to the immunization of rabbits against the pathogenic action of staphylococci (aureus?); and of Rodet and Courmont (1892), who have studied the chemical products of the staphylococci and have tested their pathogenic action upon dogs and rabbits.

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XVIII.

SYMPTOMATIC ANTHRAX.

This disease of cattle is popularly known as "black leg," or "quarter evil," and is described by German authors under the name of *Rauschbrand*—French, "*charbon symptomatique.*" The disease prevails during the summer months in various parts of Europe, and to some extent in the United States. It is characterized by the appearance of irregular, emphysematous swellings of the subcutaneous tissues and muscles, especially over the quarters. The muscles in the affected areas have a dark color and contain a bloody serum in which the bacillus is found to which the disease is due. This is an anaërobic bacillus which forms large oval spores.

The etiology of the disease was first clearly established by the researches of Arloing, Cornevin, and Thomas (1880 to 1883), and subsequent researches have shown that immunity may be produced in susceptible animals by protective inoculations.

The disease causes considerable losses among cattle in certain sections. Horses do not contract it spontaneously, and when inoculated with a culture of the bacillus present only a limited local reaction. Swine, dogs, rabbits, fowls, and pigeons have but slight susceptibility. The researches of the authors above mentioned have shown that the virulence of a culture is greatly increased by adding to it twenty per cent. of lactic acid. The guinea-pig is

the most susceptible animal and succumbs in from twenty-four to thirty-six hours when inoculated subcutaneously with a small quantity of a pure culture. According to Kitasato cultures in a bouillon made from the flesh of the guinea-pig soon lose their virulence, while cultures in solid media preserve their virulence for a long time. Cultures are readily attenuated by heat, according to the method of Toussaint and Chauveau—exposure to a temperature of 42° to 43° C. in the absence of spores. The spores are attenuated by exposure for several hours to a temperature of 80° to 100° C. Arloing, Cornevin, and Thomas recommend for the production of immunity in cattle inoculation with a dried powder of the muscles of animals recently dead from the disease. This is attenuated by heat. According to Kitt the muscles should first be dried at 32° to 35° C. and then powdered. Two "vaccines" are prepared from this powder—a strong vaccine by exposure to a temperature of 85° to 90° C. for six hours, and a weaker vaccine by exposure for the same time to a temperature of 100° to 104° C. (dry heat). An inoculation is first made with the weaker vaccine which gives rise to a local reaction of moderate intensity. Later a second inoculation is made with the stronger vaccine, after which the animal is immune from the pathogenic action of the most virulent material. Immunity may also be secured by intravenous injections; or, in guinea-pigs, by inoculations with cultures which have become attenuated by being kept a few days, or by exposure to a temperature of 42° to 43° C.; or by inoculation with a very small quantity of a pure culture; or by inoculations with filtered cultures (Roux and Chamberland); or with cultures sterilized by heat (Kitasato). A non-fatal and protective local infection may also be pro-

duced in cattle by inoculations with virulent material made into the extremity of the tail. Roux has claimed that animals which have an acquired immunity against symptomatic anthrax are also immune against the pathogenic action of the bacillus of malignant œdema; but Kitasato was unable to confirm this.

Strebel, in 1885, published the results of protective inoculations made in Switzerland in 1884. The inoculations were made in the end of the tail with two "vaccines," with an interval between the two of from nine to fourteen days. The vaccines were prepared by exposure to heat, as above recommended by Arloing, Cornevin, and Thomas. The most favorable season for inoculations was found to be the spring, and the most favorable age of cattle for inoculation from five months to two years.

In seven Swiss cantons 2,199 cattle were inoculated; 1,810 inoculations were made among animals which were exposed in dangerously infected pastures. Of these but two died, one two months and the other four months after the protective inoculations. Among 908 inoculated cattle, which were pastured with 1,650 others not inoculated, the mortality was 0.22 per cent., while the loss among the latter was 6.1 per cent. The following year (1885), according to Strebel, the number of inoculations, exclusive of those made in the canton of Bern, was 35,000. The losses among inoculated animals are reported as having been about five times less than among those not protected in this way. In the canton of Bern, in the same year, according to Hess, 15,137 cattle were inoculated by thirty-eight veterinarians—12,190 of these were pastured in dangerously infected pastures. The results are said to have been favorable to the method, but the abstract at hand does not give the precise figures.

In 1887 Kitt reported the results of his investigations, which were confirmatory of those previously published by Arloing, Cornevin, and Thomas, and also of a new method of inoculation, which presented the advantage that a single inoculation was sufficient to confer immunity. This was made in the region of the shoulder with a vaccine somewhat stronger than that employed by the French bacteriologists, but which was found to be without danger for cattle. It produced only a slight local effect. His vaccine was prepared by heating the moistened flesh of an animal just dead from the disease to 85° to 90° C. for six hours. This did not kill the spores present, but caused a sufficient attenuation in their virulence.

In a later communication (1888) Kitt recommends that the flesh of the diseased animal be first dried and pulverized, and then subjected to a temperature of 100° C. in streaming steam for six hours, after which it is to be again dried and used for subcutaneous inoculations. The dose is from 5 to 15 ctgr.

Roux (1888) has shown by experiment that sterilized cultures of the bacillus, which have been exposed to a temperature of 115° C., when injected in doses of 40 c.c., three times repeated, into the cavity of the abdomen of guinea-pigs, cause these animals to be completely immune against the most virulent material. Cultures from which the bacilli have been separated by filtration are still more active. And immunity could easily be conferred by the subcutaneous inoculation, in guinea-pigs, of 1 c.c. of the filtrate from the serum obtained from the œdematous tissues of a diseased animal.

Schuhanka (1888) has reported the results of inoculations made in the dukedom of Salzburg during the year

1887. In all 2,596 cattle were inoculated once, and 2,472 twice, with an attenuated virus, in forty-seven different parishes. Most of these were from six months to a year old. No losses occurred as a result of the inoculations. During the summer of 1887 the 2,472 cattle which had been twice inoculated were associated in infected pastures with 3,561 unprotected cattle. The loss among the former was 8, = 0.32 per cent., among the latter it was 235, = 6.31 per cent.

Strebel reports similar results, in 1887, in the canton Freiburg, where 1,725 cattle which had been inoculated suffered a loss of 0.23 per cent., and 1,945 associated cattle a loss of 5.28 per cent.

Lydtin (1892) reports the results of inoculations made in five districts (*Amtsbezirken*) in Baden during the years 1886–91 : 2,797 cattle were inoculated with a loss of three only as a result of the inoculation. None of the inoculated cattle subsequently contracted the disease.

In the *Bulletin of the Central Society of Veterinary Medicine* of France (1892) Guillod and Simon give the results of 3,500 inoculations made since 1884. The mortality among cattle in the region where these inoculations were practised had been from ten to twenty per cent., but fell to 0.5 per cent. among the inoculated animals.

The authors last named prefer inoculations in the region of the shoulder to the plan first practised of inoculating in the end of the tail. Strebel also (1892) advocates this method, which is quickly carried out and attended with but little loss. According to Strebel the loss among 13,022 inoculated in this way only amounted to five, while the loss among animals inoculated by the old method was twice as great.

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XIX.

TETANUS.

The experiments of Kitasato (1889) show that pure cultures of the tetanus bacillus injected into mice, rabbits, or guinea-pigs produce typical tetanic symptoms and death. As the presence of this bacillus at the seat of injury, in cases of tetanus in man, has now been demonstrated by numerous observers, there is no longer any question that tetanus must be included among the traumatic infectious diseases, and that the bacillus of Nicolaier and of Kitasato is the specific infectious agent. Kitasato's recently published experiments (1890) show that cultures of the tetanus bacillus which have been sterilized by filtration through porcelain produce the same symptoms, and death, in the animals mentioned, as result from inoculation with cultures containing the bacillus. It is evident, therefore, that death results from the action of a toxic substance produced by the bacillus. This is further shown by the fact that the bacillus itself cannot be obtained in cultures from the blood or organs of an animal which has succumbed to an experimental inoculation with an unfiltered culture; but the blood of an animal killed by such an inoculation contains the tetanus poison, and when injected into a mouse causes its death with tetanic symptoms.

When a platinum needle is dipped into a pure culture of the tetanus bacillus, and a mouse is inoculated with it subcutaneously, the animal invariably falls sick within twenty-four hours and dies of typical tetanus in two or three days. Rats, guinea-pigs, and rabbits are killed in the same way by somewhat larger quantities—0.3 to 0.5 c.c. (Kitasato). Pigeons are very slightly susceptible. The tetanic symptoms are first developed in the vicinity of the point of inoculation ; if the animal is inoculated in the posterior portion of the body the hind legs first show tetanic contraction, if in the forepart of the body the muscles of the neck are first affected. At the autopsy there is a certain amount of hyperæmia at the point of inoculation, but no pus is formed ; in inoculations with garden earth, or accidental inoculations in man, pus is commonly found in the vicinity of the inoculation wound. The various organs are normal in appearance. Kitasato says that he has not been able to demonstrate the presence of the bacillus or of spores in the spinal marrow, the nerves, muscles, spleen, liver, lungs, kidneys, or blood from the heart ; nor has he been able to obtain cultures from the various organs. In mice which were inoculated at the root of the tail Kitasato was able to demonstrate the presence of the bacilli at the point of inoculation by the microscopical examination of an excised piece of the tissues for eight to ten hours after the inoculation ; later than this they were not found. In pus from the inoculation wounds of men and animals accidentally infected the bacilli are present, but the formation of spores does not always occur. According to Kitasato, the sooner death has occurred after accidental inoculation the less likely are spores to be found in the rods, but from pus in which no spores are seen cultures

of the bacillus may be obtained in which spores will develop in the usual manner.

Guinea-pigs are even more susceptible to the tetanus poison than mice, and rabbits less so. The amount of filtrate from a slightly alkaline bouillon culture required to kill a mouse is extremely minute—0.00001 c.c. (Kitasato). The tetanic symptoms are developed within three days; if the animal is not affected within four days it escapes entirely. The tetanus poison is destroyed by a temperature of 65° C. maintained for five minutes, or 60° for twenty minutes, or 55° for an hour and a half; in the incubating oven at 37° C. it gradually loses its toxic potency; in diffuse daylight, also, its toxic power is gradually lost; in a cool, dark place it retains its original potency indefinitely; in direct sunlight it is completely destroyed in from fifteen to eighteen hours; it is not injured by being largely diluted with distilled water; it is destroyed in an hour by hydrochloric acid in the proportion of 0.55 per cent.; terchloride of iodine destroys it in the proportion of 0.5 per cent.; cresol in one per cent.—one hour's exposure. In general it is destroyed by acids and by alkalies. Blood-serum from cattle, horses, sheep, rabbits, rats, or guinea-pigs does not modify its toxic properties.

Brieger (1886) first succeeded in obtaining from impure cultures of the tetanus bacillus a crystallizable toxic substance, called by him *tetanin*, which was found to kill small animals in very minute doses and with the characteristic symptoms of tetanus. More recently Kitasato and Weyl have obtained the same substance, by following Brieger's method, from a pure culture of this bacillus. From a bouillon made from 1¼ kilogr. of lean beef, with the addition of 25 gm. of peptone, they obtained

1.7118 gm. of hydrochlorate of tetanin. This proved fatal to white mice in six hours in the dose of 0.05 gm., and a dose of 0.105 gm. caused characteristic tetanic convulsions and death within an hour. The bacteriologists last named also obtained from their cultures the *tetanotoxin* of Brieger. Two mice were inoculated subcutaneously with 0.003 gm. of this substance; one died at the end of five hours without the development of tetanic symptoms; the other survived. In addition to these substances, indol, phenol, and butyric acid were demonstrated to be present in cultures of the tetanus bacillus.

The more recent researches of Brieger and Fränkel, and of Kitasato, show that the toxic ptomaïne discovered by Brieger in 1886 is not the substance to which cultures of the tetanus bacillus owe their great and peculiar pathogenic power. The distinguished German chemist and his associate have succeeded in isolating from tetanus cultures a *toxalbumin* which is far more deadly than tetanin.

Brieger and Cohn in more recent investigations (1893) relating to the toxic products of the tetanus bacillus have arrived at the following results: The cultures were made in veal bouillon containing one per cent of peptone and one-fifth per cent. of chloride of sodium. Large quantities of the cultures in this medium were filtered through porcelain filters. The active substance was precipitated from the filtrate by means of a saturated solution of ammonium sulphate. By adding this salt in excess the precipitate is made to rise to the surface and is skimmed off with a platinum spatula. The liquid is removed by placing this upon porous porcelain plates and the crude toxin is dried in a vacuum. It still contains 6.5 per cent. of ammonium sulphate. The tetanus bouillon after filtra-

tion is said to be fatal to mice in the dose of 0.00005 c.c. A litre of this bouillon gave about one gramme of the dried precipitate, which produced characteristic tetanic symptoms and death when injected into mice in the dose of 0.0000001 gm. Kitasato in his experiments had previously obtained a tetanus bouillon which was five times as toxic as that used by Brieger and Cohn in their experiments, and which killed mice in the dose of 0.00001 c.c. The dried precipitate obtained by Brieger and Cohn contained various impurities, including a certain amount of ammonium sulphate, but was found to kill susceptible animals in the proportion of 0.0000066 gm. per kilogm. of body-weight.

It was purified without loss of toxic power by placing it in a dialyzer in running water for from twenty-four to forty-eight hours, after which it was dried *in vacuo* at 20° to 22° C. The purified toxin as thus obtained had a slightly yellowish color, and was in the form of transparent scales, which were odorless, tasted like gum acacia, and were easily soluble in water. The chemical reactions of this purified toxin, according to Brieger and Cohn, show that it is not a true albuminous body. When injected beneath the skin of a mouse weighing 15 gm., in the dose of 0.00000005 gm., it causes its death, and one-fifth of this amount gave rise to tetanic symptoms from which the animal recovered after a time. The lethal dose for a man weighing 70 kilogm. is estimated by the authors named to be 0.00023 gm. (0.23 milligm.). Comparing this with the most deadly vegetable alkaloids known it is nearly six hundred times as potent as atropin and one hundred and fifty times as potent as strychnin.

Fermi and Pernossi (1894), as a result of an elaborate research, have determined many of the chemical charac-

ters of the tetanus toxin. When in solution it is destroyed by a comparatively low temperature (55° C. for one hour) and by exposure to direct sunlight, but the dry powder resists a temperature of 120° C. It has not the properties of an alkaloid, as it is not dissolved by any of the usual solvents of these bodies—the only solvent thus far discovered is said to be water. It resembles the albumins and peptones in its failure to pass through a dialyzing membrane. The authors last referred to conclude their summary of results as follows:

"The appended table shows that the tetanus poison, like that of diphtheria, in its behavior as regards the action of light, heat, chemical agents, and dialysis, as also its solvents, the agents which precipitate it, and its action upon living animals, closely resembles the poisons of serpents (Naja tripudians, Crotalus, etc.). As to the chemical nature of this group of substances we can at present only say that they rather have the characters of colloidal substances than otherwise, and more nearly resemble the albuminoid bodies than the bases. We do not, however, reject the very probable hypothesis that these toxins are acids or bases, or other very unstable, peculiar substances which are closely united with colloidal substances, as is the case, for example, with the alkali and acid albumins and so many other albuminous bodies."

While the exact nature of the toxic substance contained in tetanus cultures has not been determined we probably cannot, at present, do better than to continue to speak of it as a "toxalbumin."

Kitasato (1891) was not able to produce immunity in mice by inoculations with minute doses of the poison, or with a filtrate which had been exposed to various degrees of temperature by which its activity was diminished or

destroyed. But immunity lasting for about two months was produced in rabbits by inoculating them with the filtrate from a culture of the tetanus bacillus, and subsequently, in the same locality, with 3 c.c. of a one per cent. solution of terchloride of iodine; this last solution was injected subcutaneously in the same dose at intervals of twenty-four hours for five days. Of fifteen rabbits treated in this way six proved to be immune against large doses of a virulent culture of the tetanus bacillus. The same treatment was not successful in producing immunity in mice or guinea-pigs, but the important discovery was made that a small quantity of blood (0.2 c.c.) from an immune rabbit, when injected into the abdominal cavity of a mouse, gave it immunity from the effects of inoculations with the tetanus bacillus. Moreover, mice which were first inoculated with a virulent culture of the bacillus, and, after tetanic symptoms had appeared, received in the cavity of the abdomen an injection of blood-serum from an immune mouse, were preserved from death. The power of the blood of an immune animal to neutralize the tetanus poison was further shown by mixing the filtrate from a virulent culture with blood-serum from an immune animal and allowing it to stand for twenty-four hours; a dose three hundred times greater than would have sufficed to kill a mouse proved to be without effect after such admixture with blood-serum; as before stated, the blood-serum of animals which are not immune has no effect upon the poison. The duration of immunity induced in this way was from forty to fifty days. Blood-serum from an immune rabbit, preserved in a cool, dark room, retains its power of neutralizing the tetanus poison for about a week, after which time it gradually loses it. Having found that chickens have a natural immunity

against tetanus, Kitasato made experiments to ascertain whether their blood-serum would also neutralize the tetanus poison; the result was negative.

That the tetanus poison is present in the blood of individuals who die from tetanus has been proved by Kitasato by injecting a small quantity (0.2 to 0.3 c.c.) of blood from the heart of a fresh cadaver into mice; the animals develop typical tetanic symptoms and die in from twenty hours to three days.

Tizzoni and Cattani have (1891) reported results similar to those obtained by Kitasato. By repeated inoculations with gradually increasing doses of the tetanus poison they succeeded in making a dog and two pigeons immune, and found that blood-serum from this immune dog, in very small amount, completely destroyed the toxic power of a filtrate from cultures of the tetanus bacillus—one to two drops of serum neutralized 0.5 c.c. of filtrate after fifteen to twenty minutes' contact. They also ascertained that small amounts of blood-serum from this immune dog injected into other dogs or white mice produced immunity in these animals; but they were not able to produce immunity in guinea-pigs or rabbits by the same method.

In a later communication (May, 1891) Tizzoni and Cattani give an account of their experiments made with a view to determining the nature of the substance in the blood-serum of an immune animal which has the power of destroying the toxalbumin of tetanus—"tetanus antitoxin." They found, in the first place, that this antitoxin in blood-serum is destroyed in half an hour by a temperature of 68° C.; further, that it does not pass through a dialyzing membrane; that it is destroyed by acids and alkalies. As a result of their researches they conclude

that it is an albuminous substance having the nature of an enzyme.

Vaillard has succeeded in producing immunity in rabbits by repeated injections into the circulation of filtered cultures—in all 20 c.c.—which had been exposed for one hour to a temperature of 60° C. At a temperature of 65° C. both the toxic and the immunizing action is destroyed.

Behring (1892) gives the following account of a method which he has successfully employed for producing immunity in large animals—especially in horses: A culture of the tetanus bacillus is made, in bouillon, of such toxic potency that 0.75 c.c. will kill a rabbit in three or four days. To 200 c.c. of this culture he adds carbolic acid in the proportion of 0.5 per cent. for the purpose of preserving it. The horse first receives a subcutaneous injection of 10 c.c. of this culture fluid to which terchloride of iodine ($I Cl_3$) has been added in the proportion of 0.25 per cent.; at the end of eight days 20 c.c. of the same mixture are given; again in eight days the dose is repeated; then, after an interval of three days, 30 c.c. of the same mixture. Following this, at an interval of eight days, he gives two injections of 30 c.c. each of a mixture containing one-half the quantity of $I Cl_3$ (0.175 per cent.). The proportion of the iodine terchloride is then reduced to 0.125 per cent., and two doses of 20 c.c. each are given. Finally the culture-fluid is administered in the dose of 0.5 c.c., and this dose is doubled every five days. Before giving the first dose of culture-fluid without the addition of $I Cl_3$, the immunizing value of the blood-serum of the horse is tested upon mice, and if it falls below 1 to 100 a dose of 0.25 c.c. is given instead of the larger dose (0.5 c.c.) above mentioned.

Schütz (1892) has applied Behring's method to a considerable number of horses and sheep, and arrives at the conclusion that it is a reliable method of protecting these animals against infection with living tetanus bacilli and against the toxic action of filtered cultures ; that the degree of immunity and the antitoxic power of the blood-serum increases as larger doses are gradually given. According to Behring the immunizing value of blood-serum from a horse treated in this way is very high. As tested on mice it may be 1 to 200,000, or even more. According to his calculations a serum having a value of 1 to 100,000, as tested on mice, should be given to a man weighing 50 kilogr. in the quantity of 50 c.c., given in the course of two days, in order to insure immunity.

The same author in a subsequent paper (1892) gives details as to the method of estimating the therapeutic value of serum from an immune animal. He first calls attention to the fact that the only reagent by which the antitoxic potency of this serum can be tested is the body of a living animal. The test animal selected is the white mouse. When the statement is made that a serum has the value of 1 to 1,000,000, he means that by an experimental test, made upon white mice, it has been ascertained that these animals are protected from fatal infection with the minimal lethal dose of a tetanus culture by the use of 0.00002 gm. of the serum for a mouse weighing 20 gm. For the cure of tetanus in the mouse, after the first symptoms of the disease have appeared, a dose at least one thousand times as great as the immunizing dose is required, and the more advanced the progress of the case the greater the dose must be. A serum of the strength above indicated, if used for the treatment of a case of tetanus in man, should, according to Behring, be

employed in doses amounting altogether to at least 100 c.c.—given inside of twenty-four hours in doses of 20 c.c. each. For persons sixteen years old he would give doses of 10 c.c., and for children under six, 5 c.c. at a dose. The serum of this strength which he had prepared for testing its curative value on man was preserved by the addition of 0.5 per cent. of carbolic acid.

Rotter (1892) reports a case successfully treated by Behring's serum. In all 250 c.c. was administered subcutaneously. The case was not, however, one of the most severe forms of the disease.

Brieger and Ehrlich (1892) have succeeded in immunizing goats by means of gradually increasing doses of a culture of the tetanus bacillus in thymus bouillon. The amount given at first was 0.2 c.c., and this was gradually increased to 10 c.c. At the end of thirty-seven days the animal was found to be immune against virulent cultures, and the important fact was demonstrated that the immunizing substance (antitoxin) was present in its milk. A mouse which received 0.1 c.c. of the milk of this goat in the peritoneal cavity proved to be immune against infection as a result of inoculation with a tetanus culture. The immunizing value of the milk from this goat was found to be 1,600. That is, a dose of 0.2 c.c., which was equal to 1 to 100 of the body-weight of the animal, protected a mouse from sixteen times the fatal dose of a tetanus culture. After precipitation of the casein the milk still preserved its antitoxic power unimpaired, and by concentrating it *in vacuo* a fluid was obtained which proved to have an immunizing value of 5,000.

In a later communication (1893) Brieger and Cohn give the results of additional experiments with the milk

of immunized goats. Animals were chosen which were two or three years old and had given birth to young a few weeks before the inoculations were commenced. It having been previously shown by Ehrlich that the precipitated tetanus toxin from cultures could be successfully used to immunize guinea-pigs, the same substance was employed in these experiments. The treatment was commenced with a dose of 0.00001 gm., which was carefully increased to 0.00007 gr., the injections being made at intervals of four days. But this proved to be too much, and the animal died of typical tetanus after the last dose. In a subsequent experiment Brieger and Cohn succeeded in immunizing a goat in a month and a half so that the animal finally withstood a dose of 0.06 gm., but this animal ceased to give milk, became anæmic, and finally died.

The authors therefore resorted to a different method which had previously been successfully employed by Ehrlich, Behring, and others. Cultures of the tetanus bacillus in bouillon were heated to 65° C. for half an hour, and then used for immunizing two goats. After five weeks' treatment the animals resisted doses of the precipitated toxin, which were gradually increased to 10 gm., at which time the treatment had been carried on for nearly six months and the antitoxic value of the milk was found to be 90,000 immunization units.

The method of determining antitoxic values adopted by Brieger and Cohn is the following: They had found by carefully conducted experiments that their precipitated toxin (*Rohgifte*) killed a mouse weighing 20 gm. in the dose of 0.0000003 gm., but failed to kill when injected in the dose of 0.0000002 gm. The first-mentioned dose was therefore accepted as the minimum fatal dose for an

animal weighing 18 to 20 gm., and the object in view was to find the minimum amount of milk required to prevent the toxic action of such a dose.

The antitoxin was obtained from the goat's milk by precipitation with ammonium sulphate, thirty-two per cent.; the precipitate was again dissolved and treated with a solution of basic acetate of lead; this salt does not precipitate the antitoxin when the solution is slightly alkaline; the voluminous precipitate produced by the lead acetate is filtered out and repeatedly washed with water; the filtered fluid and wash-water are again treated with ammonium sulphate, added to saturation, and the resulting precipitate is dissolved in a small quantity of water; a precipitate is again obtained by saturation with ammonium sulphate, and this is dried upon porcelain plates in a vacuum. The ammonium sulphate remaining could not be removed by dialysis, as experiment showed that a considerable loss of the antitoxin occurred in a dialyzer placed in running water. But by shaking up the dry powder in chloroform the heavy salt sank to the bottom and the purified antitoxin floated on the surface and could be recovered by skimming it off. The powder thus obtained consisted of a mixture of various substances, including the antitoxin, and when obtained from milk having an antitoxic value of 90,000 it was found to have a value of 25,000,000 immunization units. By further purification a still higher value was obtained (55,000,000). In experiments on mice a dose ten thousand times as great as was necessary to produce immunity proved to exercise a curative power—*i.e.*, a dose of 0.02 gm. for a mouse weighing 20 gm. saved it from being killed by double the minimum fatal dose of the tetanus toxin, after tetanic symptoms had been developed.

Reference has been made to the production of immunity by the use of cultures made in thymus bouillon. This was made known through the experiments of Brieger, Kitasato, and Wassermann (1892). The thymus bouillon is made from the thymus glands of calves, which are chopped fine in a hash-machine and covered with an equal volume of distilled water. The mixture is stirred for some time and then placed in an ice-chest for twelve hours; the liquid is then obtained by filtration through gauze with pressure—by means of a flesh-press machine. A turbid, slimy fluid is thus obtained, which is diluted with an equal volume of water and made slightly alkaline by the addition of soda solution. It is then sterilized at 100° C. for fifteen minutes. As a result of this the liquid has a grayish-brown color, and some large flocculi in suspension, which are removed by passing it through fine linen. The fluid is then of a milky opalescence. It is next placed in test tubes and again sterilized. The tetanus bacillus when cultivated in this medium does not form spores, and the toxic potency of the culture is very much reduced—1 to 5,000 to 1 to 3,000 of the toxic potency manifested by cultures of the same bacillus in ordinary media. Inoculations with cultures in thymus bouillon were found to kill mice in the dose of 0.5 c.c., while smaller amounts failed to kill and caused the animals to be immune. A culture in ordinary bouillon was fatal to mice in the dose of 0.001 c.c.

Experiments on rabbits (thirty-five) gave a uniformly successful result in immunizing these animals. Immunity was established in the course of two weeks, and the blood-serum of these animals tested on mice showed an antitoxic value of 1,000.

Reference has already been made to the earlier re-

searches of the Italian investigators, Tizzoni and Cattani. These have been followed by additional investigations, the results of which have been reported in numerous published papers. The authors named have ascertained that when kept in a cool place (15° to 25° C.) the blood-serum of immune rabbits retains its antitoxic power for several months, and the antitoxin, obtained by precipitation with alcohol, kept in a dry condition for more than ten months, was found to preserve its original activity.

Having succeeded in their earlier experiments in immunizing rabbits and dogs, Tizzoni and Cattani (in 1893) proceeded to experiment upon horses, and were equally successful with these animals. As a result of numerous injections with an attenuated virus, continued for a period of ninety-seven days, they established an immunity which was tested by inoculating the animal with 10 c.c. of a gelatine culture, of which 1-200th part of a drop killed a white mouse. The antitoxic value of the blood-serum of this horse was 1 to 5,000,000—*i.e.*, 1 gm. of this serum would immunize 5,000,000 gm. of mice, or 250,000 mice weighing 20 gm. each. In a later communication (1894) the authors named report that after freely bleeding immunized horses, and allowing them to rest for one or two months, and then again treating them with small doses of tetanus cultures, the blood-serum soon becomes as active as before the bleeding. The greatest antitoxic power was manifested from twenty to twenty-three days after the completion of the protective inoculations, and a serum was obtained possessing a value of 1 to 10,000,000. According to the authors named the precipitated (by alcohol) and purified antitoxin from such a serum, judging from their experiments on lower animals, should cure a case of tetanus in man in the dose of 40 to 50 ctgr.

The authors last mentioned have reported (1892) that the young of immune parents have a certain degree of inherited immunity. And the more recent experiments of Ehrlich and Hübener have confirmed this so far as the inheritance of immunity from the mother (in mice) is concerned; but their results did not show any immunity in the young when only the father had been rendered immune; and the immunity inherited from the mother only lasted for two or three months after birth.

Serum-therapy in Man.

The evidence has already been presented in support of the therapeutic value of the blood-serum from animals immunized against tetanus, and of the precipitated antitoxin from such serum. It only remains to present the results of the same treatment as applied to tetanus in man. Dr. Rudolph Schwarz, Assistant to the Surgical Clinic of Padua, reported the first successful case, treated with an antitoxin prepared by Professor Tizzoni from the blood-serum of a dog which he had immunized against tetanus. The following is an abstract of the case as reported by Dr. Schwarz:

"The patient was a peasant boy, aged fifteen years, who wounded himself in the left forearm while attempting to cut a walnut which he had picked up from the ground. There was considerable hemorrhage, which was controlled by the application of a wad of spider's web and a bandage. A return of the hemorrhage induced the parents to take the boy to the Surgical Clinic at Padua. There, under appropriate treatment, the hemorrhage was arrested and the wound healed. Two weeks after receiving the wound symptoms of tetanus were developed. The patient was admitted to hospital three days later, at

which time the jaws were immovable and the muscles of the arm in a state of tetanic rigidity, while other muscles of the trunk and extremities were also involved. Treatment with chloral and warm baths was without effect in arresting the progress of the malady, and on the 16th of September, nine days after admission, the patient had eight or nine tetanic convulsions during the night, accompanied by difficult respiration and slight opisthotonos. No improvement having occurred under the treatment employed, it was decided to test the value of tetanus antitoxin, which had been sent for the purpose by Professor Tizzoni, who had obtained it from the blood-serum of a dog which had been rendered 'strongly immune' against tetanus. On the afternoon of September 18th, 15 ctgr. of this antitoxin, dissolved in water, were injected beneath the skin. The same quantity was injected the following day. On the morning of the 20th the patient was decidedly better; on the afternoon of this day he received 25 ctgr. of the antitoxin, and the same amount on the following day. After each injection there was a notable fall in the temperature. The patient continued to improve, and on the 23d all symptoms of tetanus had disappeared."

In a postscript to his communication Dr. Schwarz states that he has private information of two other patients who have recovered from tetanus under the same treatment—one in the hospital at Colle di Val d'Elsa (Tuscany), treated by Dr. Pacini; the other in the Surgical Clinic at Innsbruch, treated by Professor Nicoladoni.

Rénon (1892) reports two cases treated by subcutaneous injections of the blood of immune rabbits (one received in all 57 c.c., the other 80 c.c.). Both resulted fatally, but an amelioration of the symptoms was noted in both cases after each injection of the antitoxic serum.

Baginsky (1892) reports the case of a child, nine days

old, in which tetanus was developed as a result of infection through the navel. The case was treated by Kitasato with serum from an immune rabbit in doses of 0.1 to 0.4 c.c., repeated daily (total amount used 1.5 c.c.). The child died on the thirteenth day, and Baginsky infers that the doses employed were too small.

Additional cases have been reported as successfully treated by several Italian physicians (Casali, Finotti, Gagliardi, Pacini, Taruffi). In all of these cases the dry antitoxin supplied by Tizzoni and Cattani, and obtained by them from the serum of immunized dogs, was employed. The dry powder in the dose of 0.25 gm. was dissolved in water and injected subcutaneously. In another case reported by Tizzoni 40 c.c. of rabbit serum was used. Recovery occurred in all of the cases. In one very severe case (the tenth treated) reported by Finotti, improvement occurred after the administration of the antitoxin, but the supply was exhausted and the tetanic symptoms again became more severe ; upon the receipt of an additional supply and a renewal of the treatment improvement again occurred and the case recovered.

The eleventh successful case is reported by Dr. Gattai, of Pisa, the twelfth (1893) by Lesi, of Imola.

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XX.

TUBERCULOSIS.

The announcement of the discovery of the tubercle bacillus was made by Koch, in March, 1882, at a meeting of the Physiological Society of Berlin. At the same time satisfactory experimental evidence was presented as to its etiological relation to tuberculosis in man and in the susceptible lower animals, and its principal biological characters were given.

This achievement, the result of patient and intelligent scientific investigation, will always rank as one of the most important in the history of medicine. The previous demonstration by Villemin (1865) — confirmed by Cohnheim (1877) and others—that tuberculosis might be induced in healthy animals by inoculations of tuberculous material, had paved the way for this great discovery, and advanced pathologists were quite prepared to accept it. The more conservative have since been obliged to yield to the experimental evidence, which has received confirmation in all parts of the world. To-day it is generally recognized that tuberculosis is a specific infectious disease due to the tubercle bacillus.

As evidence of the thorough nature of Koch's personal researches in advance of his first public announcement, we give the following *résumé* of his investigations:

In nineteen cases of miliary tuberculosis the bacilli

were found in the tubercular nodules in every instance; also in twenty-nine cases of pulmonary phthisis, in the sputum, in fresh cheesy masses, and in the interior of recently formed cavities; in tuberculous ulcers of the tongue, tuberculosis of the uterus, testicles, etc.; in twenty-one cases of tuberculous—scrofulous—lymphatic glands; in thirteen cases of tuberculous joints; in ten cases of tubercular bone affections; in four cases of lupus; in seventeen cases of *perlsucht* in cattle. His experimental inoculations were made upon two hundred and seventy-three guinea-pigs, one hundred and five rabbits, forty-four field-mice, twenty-eight white mice, nineteen rats, thirteen cats, and upon dogs, pigeons, chickens, etc. Very extensive comparative researches were also made, which convinced him that the bacillus which he had been able to demonstrate in tuberculous sputum and tissues by a special mode of staining was not to be found in the sputa of healthy persons, or of those suffering from non-tubercular pulmonary affections, or in organs and tissues involved in morbid processes of a different nature.

The tubercle bacillus is a *strict parasite*, and its biological characters are such that it could scarcely find natural conditions, outside of the bodies of living animals, favorable for its multiplication. It therefore does not grow as a saprophyte under ordinary circumstances. But it has been noted by Roux and Nocard that when it has been cultivated for a time in artificial media containing glycerin it may grow in a plain bouillon of veal or chicken, in which media it fails to develop when introduced directly from a culture originating from the body of an infected animal. This would indicate the possibility of its acquiring the ability to grow as a sapro-

phyte ; and we can scarcely doubt that at some time in the past it was a true saprophyte. The experiments of Nuttall indicate that the bacillus may multiply, under favorable temperature conditions, in tuberculous sputum outside of the body. And it is extremely probable that multiplication occurs in the muco-purulent secretion which accumulates in pulmonary cavities in phthisical patients. In these cavities its development may, in a certain sense, be regarded as saprophytic, as it feeds upon non-living organic material.

Metschnikoff states that when kept at a temperature of 42° C. for some time the tubercle bacillus undergoes a notable diminution in its pathogenic power, and that when kept at a temperature of 43° to 44° C. it after a time only induces a local abscess when injected subcutaneously into guinea-pigs. The experiments of Löte also indicate that an "attenuation of virulence" has occurred in the cultures preserved in Koch's laboratory, originating in 1882 from the lungs of a tuberculous ape. The author named made experiments with cultures from this source (ninetieth to ninety-fifth successive culture), and at the same time with a culture obtained from Roux, of Pasteur's laboratory. Rabbits inoculated with cultures from the last-mentioned source developed a hectic fever at the end of two weeks, and died tuberculous at the end of twenty-one to thirty-nine days. Twelve rabbits were inoculated with the cultures from Koch's laboratory ; the injections were made either subcutaneously, into a vein, into the pleural cavity, or into the cavity of the abdomen. No elevation of temperature occurred in any of the animals, and they were found at the end of a month to have increased in weight. At the end of six weeks one of them was killed and tubercular nodules were

found in various organs. The remaining animals were killed at the end of one hundred and forty-four to one hundred and forty-eight days. The two inoculated subcutaneously presented no sign of general tuberculosis, but a small yellow nodule containing bacilli was found at the point of inoculation. Those inoculated by injection into a vein showed one or two nodules in the lungs containing a few bacilli. In Koch's original experiments rabbits were killed by intravenous inoculation of his cultures in from thirteen to thirty-one days. That this attenuation of virulence depends upon a diminished production of a toxic product to which the bacillus owes its pathogenic power appears to be very certain, in view of the fact that the late cultures in a series have a more vigorous and abundant development than the more pathogenic cultures obtained directly from the animal body.

The discovery by Koch of a *toxin* in cultures of this bacillus, which is soluble in glycerin, and which in very minute doses produces febrile reaction and other decided symptoms when injected subcutaneously into tuberculous animals, must rank as one of the first importance in scientific medicine, whatever the final verdict may be as to its therapeutic value in tubercular diseases in man.

The toxic substance contained in Koch's glycerin extract from cultures of the tubercle bacillus, now generally known under the name of tuberculin, is soluble in water, insoluble in alcohol, and passes readily through dialyzing membranes. It is not destroyed by the boiling temperature. According to the chemical examination of Jolles, the "lymph" contains fifty per cent. of water and does not contain alkaloids or cyanogen compounds. It contains albuminates, which are thrown down as a voluminous white precipitate by tannic acid,

and are redissolved by hot water containing sodium chloride and very diluted potash solution. The elementary analysis gave N 5.90 per cent., C 35.19 per cent., and H 7.02 per cent. The results obtained are believed to show that the active substance present in the lymph is a toxalbumin. In experiments made with Koch's lymph in Pasteur's laboratory by Bardach, a very decided elevation of temperature was produced in tuberculous guinea-pigs by the subcutaneous injection of 0.1 gm., and a fatal result by the injection of 0.2 to 0.5 gm. In man a decided febrile reaction is produced in tuberculous patients by very much smaller doses—0.001 c.c.

Hammerschlag, in his chemical researches, found that the tubercle bacillus yields a larger proportion of substances soluble in alcohol and ether than any other bacilli tested (twenty-seven per cent.). The alcoholic extract contains fat, lecithin, and a toxic substance which produces convulsions in rabbits and guinea-pigs. The portion insoluble in alcohol and ether contains cellulose and an albuminoid substance. No ptomaïnes were found, but a toxalbumin was isolated, which caused an elevation of temperature in rabbits of 1° to 2° C., lasting for a day or two.

Koch (1891) has given a full account of his method of preparing crude tuberculin, and also the process by which he obtains from this a tuberculin which appears to be pure, or nearly so. To obtain considerable quantities of the crude product the tubercle bacillus is cultivated in an infusion of calves' flesh, or of beef extract to which one per cent. of peptone and four to five per cent. of glycerin have been added. This culture liquid must be made slightly alkaline, and it is placed in flasks with a flat bottom, which should not be more than half filled—

30 to 50 c.c. The inoculation is made upon the surface with small masses from a culture upon blood-serum or glycerin agar. By accident Koch discovered that these masses floating upon the surface give rise to an abundant development, and to the formation of a tolerably thick and dry white layer, which finally covers the entire surface. At the end of six to eight weeks development ceases, and the layer after a time sinks to the bottom, breaking up meanwhile into fragments. These cultures, after their purity has been tested by a microscopical examination, are poured into a suitable vessel and evaporated to one-tenth the original volume over a water-bath. The liquid is then filtered through porcelain. The crude tuberculin obtained by this process contains from forty to fifty per cent. of glycerin, and consequently is not a suitable medium for the development of saprophytic bacteria, if they should by accident be introduced into it. It keeps well and preserves its activity indefinitely.

From this crude tuberculin Koch has obtained a white precipitate, with sixty per cent. alcohol, which has the active properties of the crude tuberculin as originally prepared. This is fatal to tuberculous guinea-pigs in doses of 2 to 10 milligm. It is soluble in water and in glycerin, and has the chemical reactions of an albuminous body. In preparing it one volume and a half of absolute alcohol is added to one volume of the crude tuberculin, and, after stirring it to secure uniform admixture, this is put aside for twenty-four hours. At the end of this time a flocculent deposit will be seen at the bottom of the vessel. The fluid above this is carefully poured off; and an equal quantity of sixty per cent. alcohol is poured into the vessel for the purpose of washing

the precipitate. This is again allowed to settle, and the procedure is repeated three or four times, after which the precipitate is washed with absolute alcohol. It is then placed upon a filter and dried in a vacuum exsiccator.

The "tuberculocidin" of Klebs is a purified tuberculin obtained by precipitation with alcohol. The precipitate is washed in chloroform and then dissolved in a mixture of carbolic acid and glycerin.

Bujwid (1894) prepares tuberculin as follows: He uses cultures on glycerin agar or in glycerin bouillon which have been kept at a suitable temperature for five to eight weeks. The glycerin-agar cultures are treated with distilled water by which the tuberculin is extracted. After adding the water the test-tubes are kept in a cool place for twenty-four hours, and this is repeated two or three times. The extract from the agar cultures or the bouillon cultures is then sterilized by exposure for from five to ten minutes to a temperature of 100° C.; then filtered through a Chamberland filter; then evaporated at a low temperature to a sirup-like consistence. When this crude tuberculin is dropped into ten times its volume of strong alcohol a brown precipitate is thrown down which contains the active principle. From the tubercle bacilli obtained by filtering his cultures Bujwid also obtained an active substance which in the dose of 2 milligm. caused an elevation of 2° C. in the temperature of an infected guinea-pig. This substance was obtained by digesting the bacilli for two months in glycerin and water (three per cent. of glycerin), filtering and evaporating the extract, and precipitation in six volumes of ninety-five per cent. alcohol. The precipitate when dried was in the form of a white powder.

Helman (1894) obtains tuberculin from potato cultures.

The sections of potato are neutralized by leaving them for half an hour in a solution of one-half to one per cent. of bicarbonate of soda, after which they are sterilized for twenty minutes in the autoclave at 120° C. The best results were obtained when the potatoes were wet with a five per cent. solution of glycerin. The sections of potato were placed in Petri's dishes upon blotting-paper wet with a sublimate solution, and the dishes containing the cultures were surrounded with cotton wet with the same solution. The cultures were subsequently treated with distilled water, to extract the active principle, which was also obtained from the bacilli by mixing them with glycerin in the proportion of 1 to 10.

Numerous experiments have been made with dead tubercle bacilli, as well as with the toxic products developed in cultures. Héricourt and Richet (1890) found by experiment that old cultures heated to 80° C., several days in succession, when injected into a vein in rabbits, in the dose of 10 to 20 c.c., caused the death of these animals. Smaller doses from which the animals recovered seemed to make them less susceptible to infection than control animals, but the number of experiments was too limited to establish this as a fact. In a subsequent (1891) communication the authors named claim to have succeeded in immunizing rabbits by injecting filtered and sterilized cultures of the tubercle bacillus, either subcutaneously (5 to 15 c.c.) or into a vein (20 to 40 drops). The injections were repeated every second or third day for a period of fifteen days, after which the test inoculation was made with a culture, obtained from a tuberculous cow in one series, and from tuberculous fowls in another. Four vaccinated rabbits in the first series escaped general tuberculosis, while four out of eight control animals died tuber-

culous. In the second series five vaccinated animals resisted infection and three out of four control animals died tuberculous.

De Schweinitz has recently (1894) reported the results of experiments with attenuated cultures of the tubercle bacillus, and has, apparently, succeeded in conferring immunity upon guinea-pigs by inoculations with such cultures.

Klebs (1891), in experiments on guinea-pigs and rabbits, convinced himself that the fatal result of an inoculation with tubercle bacilli (in the cavity of the abdomen or subcutaneously in guinea-pigs, and in the eye in rabbits) was greatly delayed by injections of Koch's tuberculin (0.3 to 0.5 c.c.) either before or after infection.

Baumgarten (1891), in experiments upon rabbits inoculated with tubercle bacilli in the anterior chamber of the eye, failed to obtain favorable results from treatment with Koch's tuberculin given in considerable doses (0.5 to 1 gm.) either before or after infection.

The results reported in the same year by Gramatschikoff, by Popoff, by Alexander and by Gasparini and Mercanti, were also unfavorable as regards an immunizing or curative effect from inoculations of tuberculin in rabbits. Dönitz, on the contrary, arrives at the conclusion that when early treatment is instituted iris tuberculosis may be arrested and cured, and the more recent experiments of Trudeau (1893) give support to this conclusion. Baumgarten, however, insists that the tuberculin treatment does not prevent metastasis to the lungs after inoculations in the anterior chamber of the eye.

Pfuhl (1891) treated forty-seven infected guinea-pigs and at the date of his report forty-four had died tuber-

culous, but the date of death was somewhat postponed by the treatment. The animals not treated succumbed at the end of eight weeks (average of all controls), and those treated with small doses of tuberculin lived, on the average, ten weeks. With larger doses still more favorable results were obtained—four lived on an average twelve weeks, and three were still living, eleven, fifteen, and sixteen weeks after infection, at the date of publication.

Kitasato (1892) also obtained favorable results in the treatment of infected guinea-pigs, and arrives at the conclusion that guinea-pigs which have been cured by the treatment are not susceptible to a second infection, for a certain time at least.

Bujwid (1892), in experiments upon guinea-pigs, found that infected animals which received from 0.05 to 0.1 gm. of tuberculin within three hours showed an elevation of temperature of 1.5° to 2° C. Thirteen infected guinea-pigs treated with tuberculin lived from two and a half to eight months, while all of the control animals (eighteen) died in from six to nine weeks. The animal which survived eight months was found not to be tuberculous but presented evidence of recovery from a former tuberculous process. In two rabbits inoculated in the anterior chamber the iris tuberculosis was favorably influenced by the tuberculin treatment, but general infection occurred and the animals died about the same time as the controls. Three apes were treated without any apparent result; they all died within two months after infection.

The experiments of Gramatschikoff, Czaplewski and Roloff, and of Yamagiva, published in 1892, show that the tuberculin treatment does not cure tuberculous infection in inoculated guinea-pigs and rabbits, and that the

bacilli retain their vitality in such animals in spite of the most persistent treatment.

Héricourt and Richet (1892), in experiments made for the purpose of immunizing animals against tuberculous infection, failed to obtain positive results in the most susceptible species—guinea-pigs, rabbits, and apes—but claim to have succeeded in immunizing dogs by intravenous injections of cultures of the bacillus of tuberculosis in fowls. Animals which had been so treated after an interval of two to six months received an intravenous injection of 1 c.c. of a culture of the bacillus tuberculosis from man. This was fatal to "non-vaccinated" dogs, as a rule, in about three weeks, but the "vaccinated" animals survived the injection.

The results obtained by Trudeau (1893) are of such interest that we shall quote *in extenso* what he says with reference to preventive inoculations:

"Antitubercular inoculation was first tried by Falk in 1883, and all attempts in this direction have resulted until recently in but an unbroken record of failures. In 1890 I added my name to the list of those who found it impossible to produce immunity in animals by this method. In 1890, Martin and Grancher, and Courmont and Dor, claimed to have produced in rabbits a certain degree of immunity by previous inoculation, after Pasteur's hydrophobia method, of avian tubercle bacilli of graded and increasing virulence. These vaccinations were, however, frequently fatal to the animals, and the immunity obtained was but slight. Richet and Héricourt have since claimed to produce complete immunity in dogs by intravenous inoculations of bird tubercle bacilli. These experimenters found that though harmless to the dog when first derived from the chicken, bird bacilli, by long cultivation in liquid media, become pathogenic for this animal, and by thus grading the virulence of the in-

jections complete immunity against any form of tubercular infection was produced in the dog. As yet these striking results have not been confirmed. The animals which I now present to you illustrate an attempt I have made along the same line to produce immunity in the rabbit. Cultures grown directly from the chicken's lesions in bouillon for, first, five weeks, then six months, were twice injected subcutaneously at intervals of twenty-one days in doses of 0.025 and 0.05, and a third injection of a still older culture was occasionally given. About one in four of the rabbits died within three months, profoundly emaciated, but without any visible tubercular lesions. The remaining animals recovered and were apparently in good health, when, together with an equal number of controls, they were inoculated in the anterior chamber of the eye with cultures of Koch's bacillus derived from the tuberculous lesions of the rabbit, and cultivated about three months on glycerin agar. The results of these inoculations present many points of interest. In the controls, as is usually the case, if the operation has been done carefully and aseptically, and with a moderate amount of dilute virus, two days after the introduction of the virulent material in the eye little or no irritation is observed, and little is to be noticed for two weeks, when a steadily increasing vascularity manifests itself, small tubercles appear on the iris, which gradually coalesce and become cheesy, intense iritis and general inflammation of the structures of the eye develop, the inoculation wound becomes cheesy, and in six to eight weeks the eye is more or less completely destroyed and the inflammation begins to subside. The disease, however, remains generally localized in the eye for many months, and even permanently. In the vaccinated animals, on the contrary, the introduction of the virulent bacilli at once gives rise to a marked degree of irritation. On the second day the vessels of the conjunctiva are tortuous and enlarged, whitish specks of fibrinous-looking exudation appear in the iris and in the anterior chamber, and more or less in-

tense iritis supervenes, but at the end of the second to the third week, when the eyes of the controls begin to show progressive and steadily increasing evidence of inflammatory reaction, the irritation in those of the vaccinated animals begins slowly to subside and the eyes to mend. The vascularity is less, the whitish spots of fibrinous material appear smaller, the structures of the eye become clearer, the inoculation wound is but a bluish fibrous scar, until in from six to twelve weeks, in successful cases, all irritation has disappeared and the eyes present, as in the animals I now show you, but fibrous evidence of the traumatism and the inflammatory processes which have been set up by the inoculation. In all the controls, as you see, the inoculation wound is cheesy and the cornea and iris are more or less destroyed by tubercle and cheesy areas.

"Some of the protected animals slowly relapse, and the one I now show you has small tubercles growing on the iris; but even in such eyes the entire absence of caseation is noticeable, and the disease progresses almost imperceptibly. I have repeated this experiment on three sets of rabbits with about the same results each time. The vaccinations as practised are of themselves, in some instances, fatal, but the fact remains that where recovery takes place a marked degree of immunity has been acquired. I do not lay any claim, therefore, to have produced a complete or permanent immunity by a safe method, but it seems to me that these eyes constitute a scientific demonstration of the fact that in rabbits preventive inoculation of bird-tubercle bacilli can retard, and even abort, an otherwise progressive localized tubercular process so completely as to prevent destruction of the tissues threatened, and that the future study of antitubercular inoculation may not be as entirely hopeless as it has until recently appeared."

We cannot attempt to review the extensive literature relating to the treatment of tuberculosis in man by

means of Koch's tuberculin and the various preparations obtained from cultures of the tubercle bacillus. We may, however, briefly summarize the results reported by the statement that the clinical evidence shows that in early and properly selected cases the treatment may exercise a favorable influence on the progress of pulmonary tuberculosis; but that it can by no means be accepted as a specific for this disease, and in advanced cases its injudicious use may do much harm.

It is evident that in a disease in which recovery sometimes occurs independently of treatment, and in clinical experiments upon patients who differ in their susceptibility to tubercular infection and in other important particulars, and in the absence of "controls" such as we find it necessary to employ in experimenting upon the lower animals, the reported results of any plan of treatment must be accepted with great caution.

Having this in view we may refer to the very favorable report of Schiess-Bey and Kartulis (1893). Out of forty-eight cases treated during a period of two years, sixteen are said to have been permanently cured. The authors referred to conclude from their experience that commencing pulmonary tuberculosis may be surely cured by the use of tuberculin in the course of three to four months; also that more advanced cases may be cured if the treatment is persisted in for six months to a year. Even cases having small cavities may, under exceptionally favorable conditions, be cured. But when large cavities exist, with hectic fever and night-sweats, the treatment is of no avail.

The authors referred to consider the treatment as devoid of danger when very small doses are employed at the outset, but these are not sufficient to effect a cure and must be gradually increased. Usually $\frac{1}{10}$ of a

milligr. was given at first, but sometimes 1 milligr. was given. These doses did not produce a decided reaction, and it was the aim of the authors to avoid such reaction by giving comparatively small doses at the outset and carefully increasing them. Finally, doses of 100 milligr. were reached, after which the dose was again gradually diminished to 10 milligr., and subsequently increased again to the maximum. Of the 48 cases treated 33 were pulmonary and 13 "surgical" (tuberculosis of bones and skin). In the last-mentioned group of cases the results led the authors to formulate the following conclusions: "Certain forms of bone and joint tuberculosis, as well as gland tuberculosis, are cured more quickly by the use of tuberculin in combination with surgical measures than by surgical measures alone." Another conclusion which may indicate an important element in the success ascribed to the tuberculin treatment is that "the Egyptian climate is especially favorable for the tuberculin treatment."

Escherich (1892) reports that in his experience pulmonary tuberculosis was rather unfavorably influenced by the tuberculin treatment than otherwise, and the results obtained in the treatment of tuberculous joints and glands could scarcely be considered favorable. But in the various forms of skin tuberculosis a curative effect was manifested.

Klebs (1892) reports that out of 33 cases treated by himself with tuberculocidin (Klebs's) 8 were cured and 21 improved. In 75 cases treated by his colleagues 6 were cured and 24 improved. He therefore concludes that this preparation is a very effective therapeutic agent for the treatment of tuberculosis.

Kaatzer (1891) reports 44 cases of pulmonary tubercu-

losis treated. Of these 14 died, 16 were discharged cured (?), 9 improved under treatment, 4 did not improve, and 1 remained under treatment at the date of publication. The commencing dose was from $\frac{1}{10}$ to 1 milligr.; the duration of treatment was from six to fifty-two weeks; the average quantity of tuberculin administered to each patient 1,535 milligr.

In the last published report of the Adirondack Cottage Sanitarium (1894) Trudeau says:

"The medical report continues to indicate that for a certain class of cases the best and most permanent results are obtained by the addition of treatment by modified tuberculin to the usual climatic, hygienic, and open-air method."

The use of tuberculin as a means of establishing the diagnosis of tuberculosis in cows, supposed to be infected, has proved to be of considerable value, and it is now extensively employed for this purpose. Eber, in a summary of the results reported by various authors up to the 15th of March, 1892, gives the following figures: In 446 tuberculous animals inoculated a positive reaction was obtained in 378 (84.75 per cent.). The dose given for a medium-sized animal was usually 0.4 to 0.5 c.c. of tuberculin diluted with nine to ten times its volume of carbolic acid water (0.5 per cent.). The injection was made in the side of the neck, preferably early in the morning or late in the evening. The characteristic reaction commenced in from six to eighteen hours after the inoculation, and lasted from three to twelve hours. The author named accepts an elevation from the normal temperature of 0.5° C., or more, lasting for several hours, as satisfactory evidence that the inoculated animal is infected with tuberculosis.

Johne summarizes the reports of various veterinarians in Germany, made during the year 1892. The total number of animals was 287, the dose of tuberculin administered, from 0.1 to 0.5 gm. Of the inoculated animals 140 reacted and were slaughtered; of these 121 were found at the autopsy to be tuberculous, and 19 were not; 17 reacted, but were not slaughtered; 87 did not react, but were slaughtered, of these 82 proved not to be tuberculous, and 5 (all reported by Schütz, Röckl, and Lydtin) were tuberculous.

Siedamgrotzky (1892) reports the results obtained in inoculations practised upon 259 cattle. Of these 209 reacted with a temperature of 40° C., or more, 17 with a temperature of 39.5° to 40° C., 37 with less than 39.5° C. In 197 the temperature reaction amounted to 1.5° C., or more, in 8 it was between 1 and 1.5° C., and in 54 less than 1° C. Those in the last group were considered as free from tuberculosis, and the others as probably tuberculous (197 as "very probably").

The results above referred to considered in connection with other published reports indicate that a reaction of 1° C., or more, is a pretty reliable indication that the inoculated animal is tuberculous, although not an infallible test.

Serum-therapy.

Tizzoni and Cantanni (1892) have made experiments which lead them to the conclusion that the blood-serum of immunized guinea-pigs contains an antitoxin which may be successfully used in the treatment of infected animals of the same species, but their experiments cannot be accepted as conclusive and require confirmation,

especially in view of the fact that so many bacteriologists have reported their failure to establish immunity in these animals.

Bernheim (1894), after immunizing animals by the injection of sterilized cultures of the tubercle bacillus (treated for an hour and a half at 60° C. and then filtered), used blood-serum from these animals in the treatment of tuberculosis in man. From 1 to 3 c.c. of this serum was injected every second day between the shoulders. The treatment in some cases was continued for five or six months. While the treatment did not prove to be specific, Bernheim considers the results obtained somewhat encouraging.

Quite recently (1895) Paquin has published a report of his method of obtaining an antitoxic serum and the results of treatment in a number of cases of pulmonary tuberculosis. He obtains his serum from horses immunized by "laboratory processes" not described in detail. His conclusions are stated as follows:

"1. Sero-therapy in tuberculosis has proved, so far, efficacious.

"2. Blood-serum of horses seems naturally antagonistic to the germs of tuberculosis, but cannot in its natural state serve in treatment with much good, as it is too slow and it takes enormous quantities of it, too, to produce useful results.

"3. Horse blood-serum may be rendered more strongly antagonistic to the tubercle germ by the treatment of the animals by a proper technique.

"4. A horse treated properly three months may yield serum with immunizing power that will probably prove sufficient to arrest consumption in the first stages in three or four months, and sometimes less; and in the second stage in four to six months or a year.

"5. Discrimination should be made in the selection of cases, and judgment exercised to exclude from the favorable class all those seriously complicated bacteriologically, certain cases complicated otherwise pathologically, and other patients which practice alone will suggest.

"6. Inasmuch as hospitals for consumptives are needed any way, the success has been sufficient already to warrant the hope that in the near future such institutions shall be built on modern principles of hygiene, for the exclusive treatment, of tuberculosis. Sero-therapy, with the adjunct treatments, promises better success than ever obtained before. I do not designate this serum as a *cure* or a *specific*, but a valuable new remedy."

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XXI.

TYPHOID FEVER.

The bacillus discovered independently by Koch and by Eberth (1881) and carefully studied by Gaffky (1884) is now generally recognized as the specific cause of typhoid fever. It is constantly found in the form of small, scattered colonies, in the spleen, the liver, the glands of the mesentery, and the diseased intestinal glands in fatal cases of typhoid fever, and may be obtained, by puncture, from the spleen during life. The typhoid bacillus closely resembles the common bacillus found in the intestine of healthy individuals which was first described by Escherich—*Bacillus coli communis*. But it can be differentiated from this by certain biological tests and is generally recognized by bacteriologists as a distinct species. Both the typhoid bacillus and the "colon bacillus," however, vary considerably as a result of conditions relating to their environment, and it is difficult to determine whether certain bacilli of this group, sometimes found in river-water, etc., are to be regarded as varieties of one or the other of the species mentioned or as distinct species.

Brieger (1885) found in cultures of the typhoid bacillus small amounts of volatile fat acids, and when grape-sugar has been added to the culture medium lactic acid. He also obtained a highly alkaline basic substance possessing toxic properties which he named typhotoxin

($C_7H_{17}NO_2$). This he supposes to be the specific product to which the pathogenic action of the bacillus is due. It produces in mice and guinea-pigs salivation, paralysis, dilated pupils, diarrhœa, and death.

More recent experiments by Pfeiffer (1894) lead him to conclude that the specific poison of the typhoid bacillus is not present in filtered cultures, but is closely associated with the bacterial cells. According to Pfeiffer the bacillus may be killed by a temperature of 54° C. without injury to this toxic substance. The fatal dose of the dead bacilli is from 3 to 4 milligr. per 100 gm. of body-weight, for guinea-pigs. Susceptible animals may be immunized by means of this toxic substance, and their blood is found to contain an antitoxin which has a specific bactericidal action upon the typhoid bacillus. But, according to Pfeiffer, the blood-serum of animals immunized in this way does not differ from normal serum in its action on bacillus coli communis and other species of bacteria. These results are believed, by the author referred to, to settle the question of the specific character of the typhoid bacillus, and to differentiate it from nearly allied species. The presence of a typhoid antitoxin in the blood-serum of individuals who have recently suffered an attack of typhoid fever has also been demonstrated by Pfeiffer.

Chantemesse and Widal (1888) first showed by experiment that susceptible animals could be made immune against the pathogenic action of this bacillus by the subcutaneous injection of sterilized cultures. Having found that four drops of a bouillon culture, three days old, injected into the peritoneal cavity of white mice caused the death of these animals within thirty-six hours, they proceeded to inject small quantities ($\frac{1}{2}$ c.c.) of a culture

which had been sterilized by heat, and found that after several such protective inoculations the mice no longer succumbed to infection by an unsterilized culture.

In experiments made upon rabbits, Bitter (1892) arrived at the conclusion that the immunity which he produced in these animals by the intravenous injection of concentrated sterilized (by filtration) cultures was due to the presence of an antitoxin in the blood of the immune animals. Having found that control animals were killed by intravenous injections of 1 c.c. of his concentrated solution of the products of the typhoid bacillus, he added to twice this amount of the toxic solution a certain quantity (?) of blood-serum from an immune rabbit, and injected the mixture into the circulation of rabbits with a negative result. Control experiments in which the toxic solution was mixed with blood-serum from non-immune animals showed that this had no antitoxic effect, and the animals died. Bruschettini obtained (1892) similar results in his experiments upon rabbits with cultures sterilized by heat (60° C.). He concludes from his experiments that the blood-serum of rabbits immunized in this way not only possesses antitoxic properties, but that it has greater germicidal potency for the typhoid bacillus than the blood of normal rabbits.

Stern (1892) has made experiments to determine whether the blood of recent convalescents from typhoid has greater germicidal power for the typhoid bacillus than that of other individuals. The result showed that the blood-serum from persons who had recently recovered from typhoid fever had no increased germicidal power, but rather showed diminished potency for the destruction of typhoid bacilli. But blood from a man who had suffered an attack seventeen and a half years pre-

viously was found to have unusual bactericidal power, although it did not protect white mice from typhoid infection. On the other hand, blood from recent convalescents served to immunize white mice, thus indicating the presence of an antitoxin. This is also shown by the experiments of Chantemesse and Widal (1892), who report their success in immunizing susceptible animals by injecting the blood-serum of other animals previously made immune by repeated injections of sterilized (by heat) cultures. The authors last named have also tested the blood-serum of typhoid-fever patients, of recent convalescents from the disease, and of persons who had suffered an attack some years before the experiment was made. The experiments were made upon guinea-pigs. The authors conclude that "in general the guinea-pig is immunized against the action of virulent typhoid cultures by the subcutaneous injection of a small quantity of serum of persons who have suffered an attack of the disease, no matter how remote." But this immunity was shown to be of short duration, and quite different from that induced by the injection of sterilized cultures, which does not immediately follow the introduction of the toxic substances, but requires a certain number of days for its development. The degree of immunity is said by the authors last named to depend to a considerable extent upon the dose given, and the animals treated in this way still resisted virulent cultures at the end of two months. On the other hand, injections of blood-serum from immune individuals were effective in doses of a single c.c., within a few hours, and the immunity conferred had a comparatively brief duration.

Serum-therapy.

In their experiments in the treatment of infected rabbits and guinea-pigs with serum from immune animals Chantemesse and Widal (1892) obtained favorable results when injections of 2 c.c. were made into the cavity of the abdomen within a few hours after infection. Favorable results were also obtained by injecting in the same way blood-serum from individuals who had suffered a recent or remote attack of the disease. Animals which recovered were immune against infection by the bacillus, but had no increased resistance against the toxic action of filtered cultures. In two cases in which typhoid patients in the eleventh and thirteenth day of the disease were treated with considerable doses of serum from immune animals, the progress of the disease was not arrested.

Neisser (1893) by the injection of serum from a typhoid convalescent, fourteen days after the termination of the fever, was able to protect mice from three or four times the lethal dose of typhoid cultures. He injected 1 or 2 c.c. daily for four days.

Stern (1894) has made similar and more numerous experiments upon mice and guinea-pigs, to test the antitoxic power of the serum of individuals who had suffered an attack of typhoid fever. The inoculations were made in the peritoneal cavity in the proportion of from 1 to 1 to 10 to 1 of serum and culture. In guinea-pigs the serum was injected from sixteen to twenty-four hours before infection. Positive results were obtained with serum from 6 out of 8 recent convalescents, and with 3 out of 5 individuals who had suffered an attack from one to ten years previously; and negative results with serum from two persons who had suffered attacks more than

ten years before the experiment was made. In a series of experiments with blood-serum from fatal cases of typhoid this was found to be still more potent than the serum of recent convalescents. In doses of from 0.02 to 0.05 c.c. it protected guinea-pigs from lethal doses of a virulent typhoid culture injected after an interval of twenty hours. The blood was obtained from a vein an hour after the death of the patient. In a series of comparative experiments with blood from individuals who had never had an attack of typhoid a protective influence was shown to be exercised in some cases, but larger doses were required. Stern does not attempt to explain this fact, but suggests the possibility that persons whose blood-serum showed this antitoxic power may have at some time suffered a mild and unrecognized attack of typhoid fever.

Cesaris-Demel and Orlandi (1894) have obtained results which confirm those above referred to as regards the protective and therapeutic value of serum from animals immunized against the typhoid bacillus; and have obtained similar results in experiments with bacillus coli communis. Moreover, they conclude, as a result of their experimental researches, that animals which have been immunized for the "colon bacillus" furnish a serum which has protective and therapeutic value against infection by the typhoid bacillus, and the reverse. The serum from immunized animals has been used by the bacteriologists referred to in the treatment of typhoid fever in man, and the results are thought to be favorable, although the number of cases so treated is still too small to justify any definite conclusion.

We may refer briefly, before concluding the present chapter, to recent experiments in the treatment of typhoid

fever with sterilized cultures of the typhoid bacillus grown in thymus bouillon (Fränkel) and of bacillus pyocyaneus (Rumpf). Fränkel and Manchot (1893) treated fifty-seven cases of typhoid fever in the new general hospital at Hamburg-Eppendorf with cultures of the typhoid bacillus in thymus bouillon, which had grown for three days in an incubating oven at 37° C., and were then sterilized by exposure to a temperature of 60° C. The treatment was commenced by the subcutaneous injection of 0.5 c.c. of the sterilized culture; the following day a dose of 1 c.c. was given; after this the injection was repeated every second day, and the dose was increased each time by 1 c.c. Usually there was a noticeable rise of temperature as a result of the injection, and in some cases a more or less pronounced chill. On the third day of treatment there was usually a fall in the temperature, followed by a still greater reduction on the following day. The conclusion is reached that the course of the fever is materially influenced by the treatment, that it is changed from a continued to a more or less remittent character, and that complete apyrexia occurs in a shorter time.

Rumpf (1893) was induced to test the value of sterilized cultures of Bacillus pyocyaneus, because it had been shown by experiments upon animals that such sterilized cultures exercised an immunizing influence against typhoid infection. He followed Fränkel's method, giving on the first day 0.5 c.c. of the sterilized culture, on the second day 1 c.c., on the fourth day 2 c.c., the sixth day 4 c.c., the eighth day 6 c.c. The injections were made in the gluteal region. They were commonly followed by a rise in the temperature and a subsequent fall, with diminished pulse-rate and profuse perspiration. Sometimes a

chill and a rise of temperature followed the injection. No effect was observed upon the secretion of urine or the respiration, but, as a rule, a general improvement in the feeling of the patient and a prompt disappearance of delirium, when it existed, was noted. Thirty cases were treated, with two deaths, one from pneumonia and one from intestinal hemorrhage.

In a recent communication (1894) Kraus and Buswell have reported the results of additional experiments. They treated in all twelve cases with sterilized pyocyaneus cultures; ten recovered, and two died. As a rule, the immediate result of an injection was a reduction of temperature lasting for several hours. It was found also that an increase in the number of leucocytes in the blood followed the injections, and the same result occurred when the injections of sterilized pyocyaneus cultures were made in rabbits. The conclusion is reached, however, that this method of treatment has no specific curative value.

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XXII.

YELLOW FEVER.

In the writer's report (1890) upon the results of his investigations relating to the etiology of yellow fever, his conclusions are stated as follows :

"The experimental data recorded in this report show that—

"The specific infectious agent in yellow fever has not been demonstrated. The most approved bacteriological methods fail to demonstrate the constant presence of any particular microörganism in the blood and tissues of yellow-fever cadavers.

"The microörganisms which are sometimes obtained in cultures from the blood and tissues are present in comparatively small numbers, and the one most frequently found (*Bacterium coli communis*) is present in the intestine of healthy individuals, and consequently its occasional presence cannot have any etiological import.

"A few scattered bacilli are present in the liver, and probably in other organs, at the moment of death. This is shown by preserving portions of liver, obtained at a recent autopsy, in an antiseptic wrapping.

"At the end of twenty-four to forty-eight hours the interior of a piece of liver so preserved contains a large number of bacilli of various species, the most abundant being those heretofore mentioned as occasionally found in the fresh liver-tissue, viz., *Bacterium coli communis* and *Bacillus cadaveris*.

"Blood, urine, and crushed liver-tissue obtained from a

recent autopsy are not pathogenic, in moderate amounts, for rabbits or guinea-pigs.

"Having failed to demonstrate the presence of a specific 'germ' in the blood and tissues, it seems probable that it is to be found in the alimentary canal, as is the case in cholera. But the extended researches made and recorded in the present report show that the contents of the intestines of yellow-fever cases contain a great variety of bacilli and not a nearly pure culture of a single species, as is the case in recent and typical cases of cholera."

At the time of my visit to Brazil (1887) Dr. Domingos Freire presented me with a culture of a micrococcus which he, at that time, considered the specific yellow-fever germ. This micrococcus did not at all conform with the descriptions which he had repeatedly published of his "cryptococcus xanthogenicus." It did not produce pigment, either black or yellow, was not pathogenic for small animals, except when injected in very large doses, and gave rise to no symptoms in inoculated animals which could be identified with those of yellow fever. On the contrary, it was an ordinary stapholycoccus, which corresponded in its morphology and growth in culture media with the well-known staphyloccus pyogenes albus. With reference to this micrococcus I quote from my report above referred to as follows:

"That this micrococcus bears no relation to the etiology of yellow fever is fully proved by my extended culture experiments in Havana during the summers of 1888 and 1889. In the entire series of autopsies I have made cultures from the liver, and in a considerable number from blood obtained directly from the heart, and I have not obtained this micrococcus of Freire in a single instance, although the culture medium commonly employed —flesh peptone-gelatine—is a very favorable one for the

growth of this coccus. Nor has it been found in the extended series of sections which I have made from the liver and kidney preserved in alcohol from my Havana autopsies. In one case only (Case X., 1888) I have found micrococci in sections from the kidney, but as the micrococcus of Friere, in its form and dimensions, resembles many others, it is impossible to say that this is or not the Freire coccus. The finding of micrococci in this case, however, does not invalidate the general result, which is that *micrococci are not found in the blood and tissues of yellow-fever cadavers.*"

In a paper read at the Quarantine Conference, held in Montgomery, Ala., in March, 1889, the present writer says:

"I may say before going any further that my faith in a living infectious agent as the specific cause of this disease is by no means diminished by my failure thus far to demonstrate the exact form and nature of this hypothetical 'germ.' The present state of knowledge with reference to the etiology of infectious diseases in general, and well known facts relating to the origin and spread of yellow-fever epidemics fully justifies such a belief. The *a priori* grounds for such faith I stated as long ago as 1873, in a paper published in the *American Journal of the Medical Sciences* (July, 1873); and the progress of knowledge since that date has all been in the direction of supporting this *a priori* reasoning. But yellow fever is by no means the only infectious disease in which satisfactory evidence of the existence of a living infectious agent is still wanting. In the eruptive fevers generally no demonstration has been made of the specific etiological agent—at least none which has been accepted by competent pathologists and bacteriologists. Again, in the infectious disease of cattle known as pleuro-pneumonia, notwithstanding very extended researches by competent investigators in various parts of the world, no satisfac-

tory demonstration of the germ has been made. The same is true of hydrophobia, in which disease we are able to say with confidence the infectious agent is present in the brain and spinal cord of animals which succumb to rabies; this infectious agent is destroyed by a temperature which is fatal to known pathogenic microörganisms (65° C.), and by various germicidal agents, yet all efforts to cultivate it or to demonstrate its presence in the infectious material by staining processes and microscopical examination have thus far been unsuccessful."

In a paper read before the College of Physicians in Philadelphia, in 1888, I give the following account of my investigations with reference to the inoculations practised by Dr. Domingos Freire in Brazil:

"Facts relating to the endemic and epidemic prevalence of yellow fever, considered in connection with the present state of knowledge concerning the etiology of other infectious diseases, justify the belief that yellow fever is due to a living microörganism, capable of development, under favorable local and meteorological conditions, external to the human body, and of establishing new centres of infection when transported to distant localities.

"Inasmuch as a single attack of yellow fever, however mild, protects, as a rule, from future attacks, there is reason to hope that similar protection would result if a method could be discovered of inducing a mild attack of the disease by inoculation or otherwise. . . .

"My own researches, recorded in the foregoing report, show that no such microörganism as Dr. Domingos Freire, of Brazil, has described in his published works, or as he presented to me as his yellow-fever germ at the time of my visit to Brazil, is found, as he asserts, in the blood and tissues of typical cases of yellow fever.

"There is no satisfactory evidence that the method of

inoculation practised by Dr. Domingos Freire has any prophylactic value.

"*Dr. Freire's Protective Inoculations.*—Having demonstrated that Dr. Freire's claim to have discovered the specific cause of yellow fever is without scientific foundation, it may be thought that no further demonstration is required in order to show that his preventive inoculations are without value; for these inoculations are said to have been made with cultures containing the attenuated microbe of yellow fever. These inoculations have, however, been made upon so large a scale, and the statistical results, as presented by Dr. Freire, appear so favorable to his method that it becomes necessary to analyze these statistics: and if, as he claims, they establish the fact that the mortality from yellow fever is very much less among those who have been inoculated by him than among non-inoculated persons exposed in the same way, we shall be obliged to concede the value of his method, although the *rationale* of the protective influence may not be apparent. In my detailed report I have reviewed at length Dr. Freire's statistics in the light of the facts developed by my personal researches in the city of Rio de Janeiro, where the inoculations were made. I cannot attempt to bring the evidence before you at the present time, and I have already stated to you my conclusion with reference to the matter. In support of this conclusion I shall, however, quote a few extracts from my report.

"In 1884 Dr. Freire inoculated 418 persons, whose names, ages, place of residence, and length of residence in Brazil are given in an appendix to his principal work, published in 1885. In regard to the evidence afforded by these inoculations, I have written as follows:

"Dr. Freire admits that 'during the epidemic season a great number of the vaccinated were attacked by the malady,' but claims that these attacks were of a mild character, yet he gives us the names of seven vaccinated persons who died from the disease. This list has been added to by some of Dr. Freire's *confrères*, as will be seen

by the following translation of a letter published in one of the newspapers of Rio, and bearing date, May 5, 1887. This letter is signed by Dr. Araujo Goes, at present a member of the Central Board of Health, and a gentleman whose statements are worthy of the fullest confidence:

"'My letter to the Imperial Academy of Medicine having been published, it now behooves me to publish the statistics relating to the vaccinations on Morro de Viuva.

"'One fact seems to me to be definitely demonstrated, that is the *worthlessness of Dr. Freire's vaccination*, as is well known to the medical profession of this city.

"'A year ago I wrote the following:

"'The want of skill which he displayed in his first experiments, the false conclusions which he has drawn therefrom, and the thoughtless precipitation with which he has hastened to make known incomplete results without accompanying them with a single qualifying remark vitiate all the methods to which he may hereafter resort to corroborate his statements (*Journal do Commercio*, April 20, 1883).

"'The mortality among the persons vaccinated on Morro da Viuva furnishes one more proof that I was right in saying this, as I now proceed to demonstrate.

"'There were vaccinated in this district 60 persons.

"'Sixteen removed shortly after the commencement of the epidemic, and 44 remained exposed to its influence. Of these 22 had yellow fever, 9 of whom died. . . .'

"In 1885 Dr. Freire resumed his inoculations on a larger scale, but instead of selecting unacclimated strangers, those inoculated were for the most part natives of Brazil, or Portuguese who had lived for a number of years in Rio and who had passed through one or more epidemics. A considerable number of negroes were also inoculated and included in the statistical tables. With reference to Dr. Freire's statistics for the year 1885 I quote from my report as follows:

"'Dr. Freire has omitted to state one very important fact with reference to the vaccinations practised during

the period included in this tabular statement. The date of the vaccinations is not given. Fortunately I am able to supply this omission from his journal containing the names of the vaccinated, which he kindly placed in my hands during my stay in Rio. I find from this record that the inoculations were practised as follows:

January	392
February	342
March	611
April	139
May	273
June	813
July	481

"'Now it is well known that June and July are months during which yellow fever does not prevail in Rio, and that, in fact, the month of May furnishes, as a rule, but few cases.

"'The exposure even in an epidemic year amounts to very little during the months of May, June, and July, and may be considered practically nil in a year like 1885, when the whole mortality was only 278 in a city of 400,000 inhabitants. But Dr. Freire has included in his list 1,294 persons who were vaccinated during the healthy winter months of June and July, and who presumably had been exposed during the preceding comparatively unhealthy months of January, February, March, and April. If these 1,291 individuals were protected from an attack of yellow fever by the inoculation practised in June or July, what protected them from being attacked during the preceding months when yellow fever was prevailing to some extent? . . .'

"I pass now to the year 1886, during which Dr. Freire inoculated 2,763 Brazilians and 710 foreigners, again including in his statistical tables those vaccinated after the epidemic season had passed. In reviewing these statistics I remark as follows:

"'We have quoted this last report of Dr. Freire *in extenso* in order to do him full justice by allowing him to

state his own case. We shall now proceed to show that his statistics are fallacious, and that the percentage of mortality among the vaccinated, which he finds to be ten times less than among the non-vaccinated, results from a misuse of the statistical method and from a number of factors which are favorable to Dr. Freire's statistics as he has stated them, but not to a fair test of his method of prophylaxis.

"'In the first place, we would call attention to the fact that while during the comparatively healthy year, 1885, the immunity among the vaccinated of that year is said to be complete (see report of 1885), the number of deaths during the epidemic year which followed is stated by Dr. Freire himself to have been eight. Taking all of the vaccinated of the two years, and without making any allowance for the considerable number of persons vaccinated who had, no doubt, left the city before the epidemic of 1886 occurred, Dr. Freire, with a total of 6,524 vaccinated, and a total of 8 deaths, makes the proportion one per thousand. This is equivalent at the outset to an addition of 1,476 persons to the number vaccinated, who being imaginary persons and not having been exposed to the epidemic influence simply aid in rounding up the general percentage of mortality in Dr. Freire's favor to the even figure of one per thousand. This is but one of many factors which go to make this favorable showing. Reference to Dr. Freire's MS. journals, which he kindly placed in my hands, shows that of the total number vaccinated during the two years, 4,465 were vaccinated prior to the epidemic of 1886; that is to say, before the 1st of January, 1886. How many of these left the city before the outbreak of the epidemic, how many were only temporarily in the city when vaccinated, how many died from other diseases I cannot say; but it is a significant fact that of the 3,051 vaccinated prior to August, 1885, Dr. Freire has only one fatal case to report, while out of 460 persons vaccinated in January and February, 1886, he reports 5 deaths, a mortality of more than one

per cent., which he gives as the general mortality among the non-vaccinated. This is not apparent from his own statement of the case, but is nevertheless true, as I shall proceed to show. In his report, which we have just given in full, he does not give the date of the vaccination of these individuals, but upon referring to his MS. journals for 1886 I find that No. 3 of his list, José, son of José da Costa Vieira, was vaccinated February 12, 1886; No. 4, Paschoal Ruffino, on the 6th of February, 1886; No. 5, Henri Constance, on the 1st of January, 1886; No. 6, Fernando Argenteiro, on the 20th of February, 1886; and No. 7, Antonio Saraiva, on the 12th of February, 1886. The same MS. record for 1886 shows that during these two months—January and February, 1886—the total number vaccinated by Dr. Freire was 460. That is to say, the mortality among those vaccinated during these two months was more than one per cent. On referring to the mortality list of the city for the same two months I find the total number of deaths to have been 369, which in a total susceptible population of 160,000 (Dr. Freire's estimate) would give a mortality of 1 in 436.'"

Dr. Carl Seidl, of Rio de Janeiro, who is Director of the San Sebastian Yellow Fever Hospital in that city, in a recently (1894) published communication calls attention to the fact that Dr. Freire's yellow-fever vaccinations "have been in progress since 1883 and yellow fever continues, becoming year by year more formidable and destructive, instead of being gradually stamped out as was prophesied by Freire. Statistics will never convince the bacteriologists of the world, the medical fraternity of Brazil, nor the public, of the value of these vaccinations. For where is the physician, where is the intelligent man in this town, who does not know cases and cases in which said vaccination was not only useless but perhaps harmful because it caused a false confidence? In the labora-

tories of Paris, Vienna, and Berlin are long series of pathogenic germs, some only recently announced, yet there is no culture of the xanthococcus which has been discovered for so many years and put to practical account in Rio." *

The evidence in favor of the value of the protective inoculations practised by the method proposed by Professor Carmona (1881), of the City of Mexico, is not satisfactory, and this method was soon abandoned. It consisted in the subcutaneous injection of material obtained by desiccating the urine of yellow-fever patients freely exposed to the air in shallow vessels. Carmona says, with reference to his inoculations:

"I usually place one or two centigrammes of the dry residue in a gramme of distilled water. I triturate it in such a manner that the mixture is as perfect as possible, and charging a Pravaz syringe, I make a subcutaneous injection in the right arm. The results are various, but no serious accident has ever occurred.

"I count, to-day, nearly two hundred persons inoculated, and among them several experienced, some hours after the inoculation, a febrile movement, which sometimes caused the thermometer to mount to 38.5° C. The duration of this febrile movement did not exceed twenty-four hours. The local accidents have been most varied. There was almost always tumefaction at the point of inoculation, but the extent and size of this tumefaction varied greatly. In many cases there was redness of the skin. These local phenomena lasted four or five days, but, in general, those inoculated continued about their ordinary affairs. Once only I have seen developed a phlegmon, which terminated by suppuration."

* Quoted from an editorial article in the Journal of the American Medical Association of January 5, 1895.

The "mosquito inoculations" of Dr. Carlos Finlay, of Havana, have not given results which have induced any of his medical *confrères* to adopt his method; and the facts, as stated by himself, do not appear to give any support to the supposition that these inoculations have a protective value. The method consists in allowing a mosquito to fill itself with blood from a yellow-fever patient, and, after two or three days, when it has digested this meal, applying it to the susceptible individual, who is supposed to be inoculated when the insect introduces its sting for the purpose of filling itself with blood. But there seems slight chance that such inoculation would occur even if the infectious element had been proved to be present in the blood drawn from the yellow-fever patient. For this blood is digested and passes through the alimentary canal, and the lancet and sucking apparatus of the insect would probably be pretty well cleaned during the interval between its application to the yellow-fever case and the person to be protected. Dr. Finlay says in his last published paper (1894):

"In my inoculation experiments the interval between the application of the contaminated mosquito to a susceptible person and the appearance of the first symptoms of a mild attack of the disease (when such a one did occur) has varied between five and twenty-five days, the latter term being the one that I have fixed upon, beyond which any morbid symptoms would be considered as independent of the inoculation. In the majority of cases no pathogenic effects were produced."

It is well established that the period of incubation in yellow fever is short, usually, if not always, less than five days; and there is not the slightest reason for the assumption that the so-called mosquito inoculation had anything

to do with the "acclimation fever" which a small proportion of those inoculated suffered in from five to twenty-five days after this operation. All of the inoculated remained in Havana and were exposed to the same influences which produce the so-called "acclimation fever" in other individuals not inoculated.

Dr. Finlay states his results as follows:

"Among the eighty-seven who have been under observation the following results have been recorded:

"1. Within a term of days, varying between five and twenty-five after the inoculation, *one* presented a mild albuminuric attack, and *thirteen* only 'acclimation fevers.'

"2. Among the seventy-three who did not present any distinct attack within the first twenty-five days—

40 were subsequently attacked with simple 'acclimation fever.'
5 suffered an attack of regular albuminuric yellow fever.
4 had melano-albuminuric yellow fever.
24 have never had any fever of the yellow-fever type.

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"*Two* of the melano-albuminuric, and *one* of the albuminuric, were fatal, giving a mortality of 3.87, equivalent to 3½ per cent. One occurred in 1884, and the two others in 1893."

The most significant fact in this statement is that nine of those inoculated suffered attacks of yellow fever—three of which were fatal. The fact that twenty-four out of the eighty-seven inoculated have remained in Havana without suffering an attack of yellow fever cannot be accepted as evidence that they are protected by the mosquito inoculation practised by Dr. Finlay, for it is well

known in Havana that strangers who have not been inoculated often remain in that city for many years without having yellow fever. Moreover, the forty cases of "acclimation fever," which, according to Dr. Finlay, occurred among the inoculated, belong in the same category as the thirteen cases which occurred in from five to twenty-five days after the inoculation was practised. This twenty-five-day limit is entirely arbitrary, and it is difficult to see how Dr. Finlay can attribute the so-called "acclimation fever," which occurs in less than twenty-five days, to the direct effects of his mosquito inoculations, and cases which occur after twenty-five days to a protective influence exercised by these inoculations.

As a matter of historical interest in connection with the subject of protective inoculations against yellow fever, I introduce here an account of the inoculations made in 1854 and 1855 by Dr. William Lambert de Humboldt, and those made in 1864 by Drs. Lebredo and Cisneros, of Havana.

Dr. de Humboldt claimed to be a nephew of the celebrated Alexander v. Humboldt. He asserted that he had discovered a sure means of protecting from yellow fever by inoculations with the venom of a poisonous snake, found in Mexico, but kept as a secret the precise species of snake from which his material for inoculations was obtained. His theory was based upon the fact that symptoms somewhat resembling those of yellow fever are produced by the venom of certain poisonous snakes. These symptoms are hemorrhage from the gums, slow pulse, fever, etc. The first experiments of Humboldt were made in Vera Cruz, in 1847, by authority of the government, upon condemned prisoners. According to Boudin the matter inoculated was an ounce of sheep's liver

which had been bitten by six of these poisonous serpents. This was left to undergo putrefaction before it was used for the inoculations.

A history of Humboldt's inoculations has been written by Dr. Nicolas B. L. Manzini. This is a volume of 240 pages, which was published in Paris in 1858. I shall quote from this work, which is entitled "*Histoire de l'inoculation préservative de la fièvre jaune, pratiquée par ordre du gouvernement espagnol, à l'hôpital militaire de la Havane. Rédigée par Nicolas B. L. Manzini, docteur en médecine de la Faculté de Paris, membre titulaire de la Société médicale d'émulation de Paris, médecin de l'Association de bienfaisance française de la Havane.*"

"I. In the month of October, 1854, Dr. William Lambert de Humboldt, residing then in New Orleans, wrote to General Don José de la Concha, Governor of the island of Cuba, announcing to him that he had discovered a substance, the active principle of which was the venom of an ophidian, which substance, inoculated by vaccination in persons who were strangers in the localities where yellow fever reigns as an epidemic, protected them from this terrible malady. M. de Humboldt said that during a period of nine years he had inoculated fourteen hundred and fifty-eight individuals. Of those whose history he had been able to follow he had only seen seven attacked with yellow fever, and of these only two had died. Out of three hundred and eighty-six inoculated in New Orleans he had not in any case seen yellow fever characterized by the pathognomonic symptoms—black vomit, etc. Finally, M. de Humboldt offered to apply his preservative to the Spanish troops of the island of Cuba, in a most disinterested manner.

"II. General Concha first consulted Dr. Basterreche, chief of the corps of military sanitation of the island of Cuba, with reference to this important affair, who consid-

ered it prudent to consult with some of his medical friends, who all gave a favorable opinion. Then General Concha, who at the outset took a lively interest in the question, consulted, officially, the University, submitting at the same time a memoir by M. de Humboldt relative to it. This corporation judged that the experiment was admissible and that the facts would decide the question. In consequence of this decision M. de Humboldt was invited to come to Havana. A ward in the military hospital was placed under his absolute direction. It was likewise agreed that all of the inoculated who should subsequently fall sick should be placed in his care, and that he could be assisted or replaced by persons of his selection. A commission of the University was named to follow the march of the operations and to make exact observations. It was composed of Drs. Cowley, Castroverde, and Benjumeda.

"III. M. de Humboldt had scarcely arrived in Havana when I put myself in relations with him. He appears to be thirty-five to thirty-six years old. He is blond, high, and slender. His chest, flattened in front, offers the conformation peculiar to the tuberculous. . . . Although his health is precarious he is endowed with a feverish activity and with a resistance to work which is surprising. M. de Humboldt speaks four languages, among them the Spanish and French, as perfectly as is possible for a foreigner. German is his native tongue, and he speaks, besides, English. . . .

"The special history of the phenomena of the inoculation which we are about to write is derived from seventy-nine observations collected and written out by M. de Humboldt and myself. Besides, we have drawn some information relative to the circulation and headache from one hundred and thirteen other cases, collected in the military hospital under the direction of M. de Humboldt.

"We have little to say of the character of the substance inoculated, which has precisely the appearance and the odor of the liquid residue of animal putrefaction.

1. LOCAL PHENOMENA OF THE INOCULATION.

"As soon as the inoculation is made, a crossed bandage is applied to the puncture and no further attention is paid to it. If it is examined at the end of a few moments it will be found to be surrounded with an elevation in the form of a white papule, diaphanous, and quite analogous to that produced by the puncture of a bed-bug. This phenomenon was visible at the end of five minutes or even of three. It was no longer seen at the end of twenty-four hours, of twelve, or even of ten. A sensation of tingling and numbness, a veritable phenomenon of slight anæsthesia, soon manifested itself in the forearm and lasted for a variable time; we have seen it persist until the fourth day in the case of Madame Mercedes Parodi. We have never seen any swelling of the axillary glands.

2. OUTLINE OF THE SICKNESS INDUCED.

"Syncope may occur at the moment of inoculation—this soon passes—or a nervous trembling, which is more rare but which lasts longer. The pulse is accelerated under the influence of the emotion of the moment. At the end of seven hours (all of the times which follow are the mean time deduced from the extremes) the pulse is modified in a permanent manner; it is more frequent or slower, stronger or more feeble. At the end of eleven hours there is febrile heat; at the end of fourteen, headache, thirst, loss of appetite; at the end of sixteen the face is red, the conjunctivæ injected, lachrymation. A commencement of swelling of the gums is observed and slight colicky pains are experienced, produced by the medicine which the patient commenced to take immediately after the inoculation.

"First. At the end of eighteen hours pain in the gums, which commence to be colored around the borders of the teeth; pain in the salivary glands and in the direction of the different nervous branches of the face and cranium.

Second: of nineteen hours, pains in the lower jaw in the direction of the inferior maxillary nerve, lassitude; of twenty hours, bitter taste, somnolence, œdema of the face; of twenty-two hours, feeling of constriction of the throat, without visible modification of the mucous membrane; of twenty-three hours, yellowness of the skin; of twenty-four hours, hemorrhage from the gums; of twenty-eight hours, eyes yellow, chills; of twenty-nine hours, inflammation of tonsils; of thirty hours, pain in region of kidneys; of thirty-six hours, œdema of eyelids; of thirty-eight hours, muscular and articular pains; of forty hours, toothache; of seventy-two hours, œdema of the lower lip; at different times, erotic phenomena. During convalescence, cutaneous itching; cutaneous eruptions of different kinds.

3. IMMEDIATE TREATMENT.

"As soon as the inoculation was made we administered a sirup composed as follows:

℞. Sirop. de mikania guaco.....187 gm.
Sirop. de rhubarbe.....125 gm.
Iodure de potassium..... 4 gm.
Gomme gutte..... 12 gm.
D. et M.

"This sirup was administered in the following manner: First day, a tablespoonful every two hours; second day, one every four hours; third day, one morning and evening. If the symptoms are more violent, the interval is shortened; and if that is not sufficient, it is necessary to add to the sirup an infusion of mikania, a teacupful every two hours."

We shall not attempt to follow our author in his account of the symptoms produced by the poisons of the various venomous snakes and in his attempt to show a resemblance between these symptoms and those of yellow fever. Nor shall we quote his account of the results of

the inoculations made, inasmuch as we have these from an official source in the report of Dr. Bastarreche, which, with great fairness, Dr. Manzini has included in his volume, although he insists, contrary to the opinion and figures of Dr. Bastarreche, that the inoculations were attended with a comparatively favorable result.

It will be sufficient to introduce here a single one of the tables appended to the report of Dr. Bastarreche.

TABLE NO. V.—COMPARATIVE SUMMARY.

	Not Inoculated.			Inoculated.			Ratio of Mortality.
	Attacked.	Died.	Ratio.	Number.	Attacked.	Died.	
Army.....	1,045	254	24.31	1,214	84	21	25.00
Navy.....	264	47	17.80	1,263	144	46	31.94
Total....	1,309	301	22.99	2,477	228	67	29.39

Nothing further has been heard of Dr. Humboldt's method of inoculation in Havana, and the inference is that by common consent the experiment made on so large a scale, and under such favorable conditions, is regarded as having demonstrated its inutility.

Another attempt to protect from yellow fever by inoculations is that made in 1864 by two physicians of Havana, members of the Academy of Sciences, and recorded in the "Anales" of the Academy. We quote the translation which Dr. Stanford E. Chaillé, President of the Havana Yellow Fever Commission of the National Board of Health, has introduced into his elaborate and valuable "Report:" *

* Annual Report of the National Board of Health for 1880, p. 166.

"In June, 1864, Drs. Lebredo and Cisneros, members of the Academy, and distinguished physicians, tested the prophylactic value of inoculated dew, by request of Drs. Masnata and Fraschieri, who had claimed for it protective power.

"The substance used was not, as had been generally supposed, natural dew, but an artificial dew obtained by the condensation of vapor of the atmosphere of the closed room of a yellow-fever patient, and collected on the surface of bottles containing water of a lower temperature than that of the surrounding air. After prolonged examination the following were our conclusions:

"Yellow fever is not a contagious nor an inoculable disease, hence the inoculation of dew cannot be effective. There is no such entity as the so-called 'fever of acclimation,' and it has not been proved that the ailments thus designated protect from yellow fever. The symptoms following the inoculation of 'rocio' lack the uniformity necessary to constitute a classifiable pathological condition as dependent solely on the inoculation; the very slight intensity of the phenomena discredit their identity with those of the so-called 'fever of acclimation;' in many instances no phenomena have ensued, and all the results obtained are explicable by disregard of hygienic laws. In three counter-experiments distilled water was inoculated. In one case more remarkable results ensued than in any case inoculated with 'rocio,' in a second the results were as mild, and in a third case no results at all ensued. Finally, as the result of experiments, the inoculation of 'rocio' is ineffective, and equally as negative as inoculations of black and of bilious vomit."

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